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Biological Activity and Localization of Phorate Residue Toxins in Soil-Drench Treated Chrysanthemums[1]

MYRON A. ANDERSON,[2] J. T. SCHULZ,[2] and E. T. HIBBS,[3,4] *Department of Zoology and Entomology, Iowa State University, Ames*

ABSTRACT

Cholinesterase-inhibiting residues of phorate persisted in the foliage of standard disbudded chrysanthemums, *Chrysanthemum morifolium* Ramat., 141 days after treatment. In plants removed to phorate-free soil after a 24-hour exposure to a soil application of phorate, (200 ml. of a dilution containing 0.5 oz. phorate per 100 gallons water applied per 6-inch pot) cholinesterase inhibitory residues were detectable for more than 47 days. The residues *in situ* killed melon aphids, *Aphis gossypii* Glover, 45 days following the application. Rates in excess of 4 oz. per 100 gallons caused phytotoxicity after some 20 days. Two ounces were not sufficient to control two-spotted mites, *Tetranychus telarius* (L.).

Cholinesterase-inhibiting residues of phorate were as much as 47 times greater in chrysanthemum foliage than in terminal inflorescences of the same plants.

A positive correlation was not evident in a comparison of the magnitude of phorate residues (expressed in grams of tissue required for 50% inhibition of cholinesterase) with toxicity (expressed in time required for 50% kill of melon aphids) for the first 20 days after application. Only after this time did a positive correlation appear.

The control of insects and spider mites, which are a continual menace to the production of greenhouse grown chrysanthemums, presents a problem in which the phytosystemic insecticides theoretically offer the advantages of simplicity of application, enduring toxicity, and specificity of action. Requisite to utilizing these compounds most effectually is an understanding of the toxicity pattern which develops in plants following applications.

Bowman & Casida (1957) and Metcalf et al. (1957) have reported the absorption of phorate (*O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate) from the soil by plant roots and its subsequent translocation and oxidation to powerful inhibitors of cholinesterase. The oxidative metabolites of phorate are: (I) *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorodithioate, (II) *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorodithioate, (III) *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate, (IV) *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate, (V), *O,O*-diethyl *S*-ethyl-sulfonylmethyl phosphorothiolate (Bowman & Casida 1957). Cholinesterase-inhibitory action of the metabolites increases successively from phorate through the phosphorothiolate sulfone, (V) (Bowman & Casida 1957, American Cyanamid 1957). However, the ascending order of toxicity to house flies progresses from (I) and IV) which are equally toxic, to (II), (V), and (III), respectively. Getzin (1958 a, b,) and David (1950) have reported greater plant absorption of systemic insecticides from sandy soils than from soil of high organic content. Although several annual and perennial food and forage plants have been included in studies of the phytosystemic action of insecticides, greenhouse grown chrysanthemums have not been extensively explored.

In this investigation, melon aphids (*Aphis gossypii* Glover) and two-spotted mites (*Tetranychus telarius* (L.)) were exposed to phorate-treated plants (*Chrysanthemum morifolium* Ramat.) in a study of the persistence, toxicity and localization of the biologically active phorate residues. Biological evidence of the accumulation and localization of phorate residue toxins in chrysanthemums was collated with the cholinesterase inhibitor activity of selected parts of treated plants. No attempt was made in this study to resolve chemically the identity or quantitative proportions of the oxidative metabolites present.

METHODS AND MATERIALS.—*Plant Culture.*—Indianapolis White chrysanthemums were grown as standard disbuds in a potting medium of 5 parts by volume Webster clay loam, 3 parts sand, and 2 parts peat in 6-inch pots situated on greenhouse benches filled with coarse gravel overlaid with one-fourth-inch mesh wire screen. An emulsifiable concentrate containing 8 pounds actual phorate per gallon was diluted with water to the desired concentration. Of this, 200 ml. was applied to drench the

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[2] Assistant Professor of Biology, Gustavus Adolphus College, St. Peter, Minnesota, and Assistant Professor of Entomology, North Dakota State University, Fargo, respectively.

[3] Associate Professor of Entomology, Iowa State University, Ames.

[4] The writers gratefully acknowledge the contributions of Dr. P. A. Dahm and Dr. W. H. Orgell toward the completion of these investigations. The skilled technical assistance of Mrs. Roger Pitstick, Mrs. Caesar Madamba, Mr. Lawrence Maher, and Mr. Eugene Eness is gratefully acknowledged. The phorate used in these investigations was supplied by the American Cyanamid Company Stamford, Connecticut. Yoder Brothers, Incorporated, Barberton, Ohio furnished the rooted chrysanthemum cuttings.

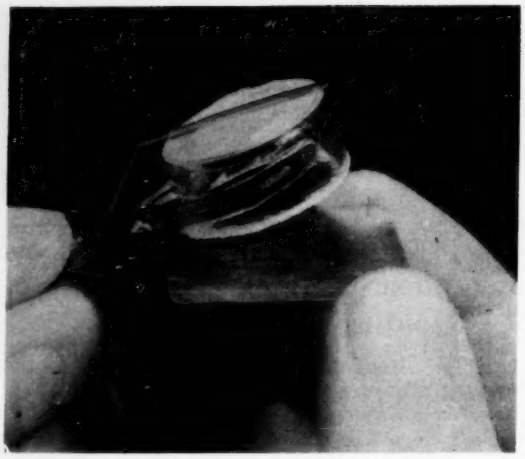

FIG. 1a.—Cage to confine melon aphids on chrysanthemum leaves.

soil of each 6-inch pot. In certain experiments the roots of a number of plants were washed free of treated soil and the plants were transplanted to phorate-free soil 24 hours after phorate application.

Aphid Tests.—Experiment 1. In order to identify the minimum dosage that would provide phorate residue toxins *in situ* capable of killing melon aphids exposed to treated plants, a known number of apterous melon aphids were placed on a single leaf of each of 10 treated plants in four replicates. The petiole was banded with tanglefoot to prevent aphids from migrating. Daily counts of living aphids were taken. When all aphids were dead a new infestation was introduced.

In a preliminary series of tests, three assays were conducted—one began at 11 days, one at 23 days and the third at 35 days following phorate application. Ten aphids were placed on a single leaf on each of 40 plants in the first assay, and 15 on a single leaf on each of 40 plants in the second and third assays. Single chrysanthemum plants (10 per treatment in four replicates) in 6-inch pots received 200 ml. of a phorate-water dilution containing either 2 oz. or 0.5 oz. phorate per 100 gallons. One group of plants was transplanted to phorate-free soil 24 hours after the soil-drench treatment. The soil was washed from the roots by agitating each root system in a large volume of water.

Experiment 2. Melon aphids were placed in cages on chrysanthemum leaves and the numbers dead and living counted at intervals until three or four values between 20% and 80% kill had been obtained. Values of per cent kill were plotted against log-time in hours and a straight line drawn to fit these points. The time needed to kill 50% of the aphids, termed the T_{50} value, was read directly from the time-mortality curve. The cages used, figures 1a and b, consisted of a section of glass tubing 18 mm. in diameter by 10 mm. high held in place on the leaf by a metal clip. A ring of velvet was glued to the base of the glass cage to prevent aphids from escaping at irregularities of the leaf surface. A removable top consisted of a metal ring covered with bolting silk. The top was held in place by a spring soldered to the base of the metal clamp. A plate of 28-gauge aluminum was attached to the lower extension of the clamp to serve as a base on the side of the leaf opposite the cage.

In each test five apterous aphids from a culture maintained on Easter lily plants were transferred with a camel's-hair brush into each cage, previously clamped to the leaf. Last instar nymphs falling into the color categories of citron yellow or pale greenish-yellow (Wall 1933) were used. Two cages were used on each of five plants per treatment. Starting time was recorded when aphids had been placed in half the number of cages used in an assay. Counts of dead and living aphids were taken at intervals, the length of which was estimated from intervals in the previous assay and from the kill observed in early counts of the current assay. At each count aphids were considered dead if they did not move after being touched with a camel's-hair brush.

Concurrent with the aphid assays of phorate residue toxins, plants of the same treated group were subjected to biochemical analysis in Experiment 4 to measure the level of cholinesterase inhibitory action.

Mite Tests.—Two-spotted spider mites also were used as indicators of toxic residues. Mites were transferred to treated plants from a culture maintained on snap beans. A bean leaf infested with mites was placed on a chrysanthemum leaf, the petiole of which had been encircled with tanglefoot; in certain experiments mites were established on plants prior to treatment. At intervals after the application of the insecticide, the living nymphs and adults present on two 12-mm.-square areas per leaf were counted. A manila card template 2.9 by 3.2 cm., from which two sample counting areas each 12 mm. square had been removed, was fastened on a metal clip and clamped to the under surface of each leaf as counts were taken.

Biochemical Analyses.—Experiment 3. Cholinesterase inhibiting residues in this series of treatments were analyzed electrometrically by the method of Hensel et al. (1954) as modified by Curry (1956) using phorate *per se* in preparing standard reference curves. Plant tissues were collected from the greenhouse, homogenized with distilled water, and filtered to yield aqueous extracts. The source

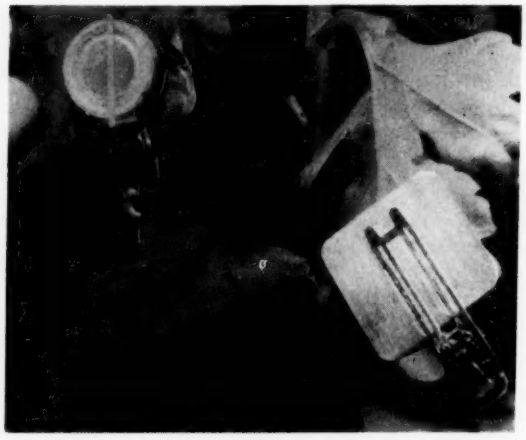

FIG. 1b.—Cages placed on chrysanthemum leaves.

of cholinesterase was pooled out-dated human blood plasma collected from local hospitals. Aliquots of the plant extract were mixed with diluted plasma and incubated 70 minutes at 37.5° C. in a constant temperature water bath. This allowed reaction of the inhibitor and enzyme. Aliquots of the partially inhibited plasma then were assayed for remaining cholinesterase activity by the electro-metric method. The decrement in pH (delta pH) during 90 minutes hydrolysis of acetylcholine was computed from pH measurements of duplicate samples. The measurements were taken with a Beckman model-G pH meter immediately upon the addition of acetylcholine and again at the end of the 90-minute interval. The decrease in pH reflects the liberation of acetic acid by the hydrolysis of acetycholine. The change in pH was directly related to the amount of cholinesterase remaining active after the initial 70-minute incubation. Extracts from phorate-free plants were subjected to the same procedure to provide control delta pH values for use in correcting for any background inhibition by untreated plant material. The residue-free extracts also were used in the preparation of standard curves for each analysis, based upon four concentrations of phorate (160, 320, 480, and 640 μg.), respectively.

Computations:

$$\text{Per cent inhibition } (\%I) = \frac{(\text{Average delta pH of control}) - (\text{Average delta pH for each treatment})}{(\text{Average delta pH of control})} \times 100.$$

The per cent inhibition was plotted against μg. of added phorate for each of the four points in the standard curve. Micrograms of phorate equivalent for a test extract was read from the 4-point standard curve. Parts per million phorate equivalent (p.p.m.) $= (w(y+a)/b)/y$ wherein W = micrograms of phorate equivalent in the aliquot of extract, y = grams of fresh plant tissue sample, a = ml. water added to y during homogenization, b = ml. of aliquot of extract taken for analysis.

Experiment 4. In a modification of Hensel's and Curry's methods a series of four aliquots (of different volumes) of the water extract of treated plants was used for each test sample. A curve was prepared in which log ml. tissue extract per 50-ml. flask was plotted against resulting per cent inhibition (usually between 20% and 90%). Experience with previous analyses suggested the approximate aliquot volumes required. A correction for apparent inhibition by untreated plant tissue for each analysis was accomplished by first establishing a 4-point curve based on untreated plant tissue to cover the range of concentrations anticipated. By reference to this curve the delta pH of untreated plant sample could be read and subtracted before calculating the per cent inhibition for the treated tissue. Plotting the logarithm of the quantity of plant extract (corrected for background inhibition) against the resulting per cent inhibition gave a sigmoidal curve from which the quantity of extract giving 50% inhibition (I_{50}) of the cholinesterase present in 5 ml. of human blood plasma could be read directly. This value divided by 4 (ratio of plant tissue to water was 1:4) represented the quantity of plant tissue in grams, required for 50% inhibition. Samples with the most powerful anticholinesterase action resulted in the smallest I_{50} values when expressed in this manner. I_{50} values do not suggest the nature of the cholinesterase inhibiting residues, and the method is adaptable to evaluating either high or low-level activity. Since each I_{50} value was computed from four points on a curve, the residue-dissipation curves, figures 3 & 4, are relatively smooth.

Concurrent with the measurement of I_{50} values aphid mortality data (Experiment 2) were taken on plants of the same treated group.

RESULTS.—*Aphid Tests.*—Experiment 1. The foliage of plants grown in soil treated 11 days previously with phorate at 2 oz. accumulated residue toxins which killed any aphids caged on the leaves within 3 days, figure 2. On the other hand, if the plants were transplanted to phorate-free soil after 24 hours and grown for 11 days without further treatment, aphids placed on the foliage were killed within 48 hours' exposure. At 23 days toxins had declined and aphids were killed only after 72 hours; at 35 days 144 hours were required to kill all the aphids applied to the foliage.

Aphids died within 72 hours on foliage of plants grown in soil which received 0.5 oz. phorate 11 days previously. Forty-five days after soil treatment some aphids survived for 10 days and were able to increase in number on the foliage of plants which had been transplanted to phorate-free soil 24 hours following the soil-drench treatment with 0.5 oz. phorate.

Within 46 days from the date of phorate application residue toxins ranged, depending upon treatment, from a highly aphicidal level to a low point approaching the threshold of toxicity. In plants remaining in contact with phorate-treated soil, the aphicidal residues from the 0.5-oz. rate were potent for at least 36 days following soil treatment. A 24-hour exposure of plants to soil receiving the same rate allowed the development of residue toxins which were relatively impotent after 24 days. This method did not provide a means of evaluating the relative potency of highly toxic levels of phorate residues. In the next experiment a method was devised which would permit, *in situ*, comparable aphid assays of highly toxic residues as well as those near the threshold of toxicity.

Experiment 2. The time required to kill 50% of the aphids (T_{50}) exposed to the foliage of treated plants was measured over a period of 65 days. On plants which received the 0.25-oz. phorate treatment (fig. 3) T_{50} values were obtained within 30 hours after aphids were caged on the foliage of treated plants 1 day following soil treatments. The time required for 50% mortality increased to 37.5 hours at 21 days following soil treatment, and finally at 35 days after treatment 76 hours were required.

T_{50} values for aphids caged on leaves of plants grown in soil that received 2 oz. phorate (fig. 4) were obtained within 18 hours 1 day following treatment. At 21 days following soil treatment 10.5 hours were required at T_{50}. The time increased to 24.5 hours on the 28th day. Thereafter T_{50} values remained relatively constant for more than 63 days. Concurrent biochemical assays are reported in Experiment 4.

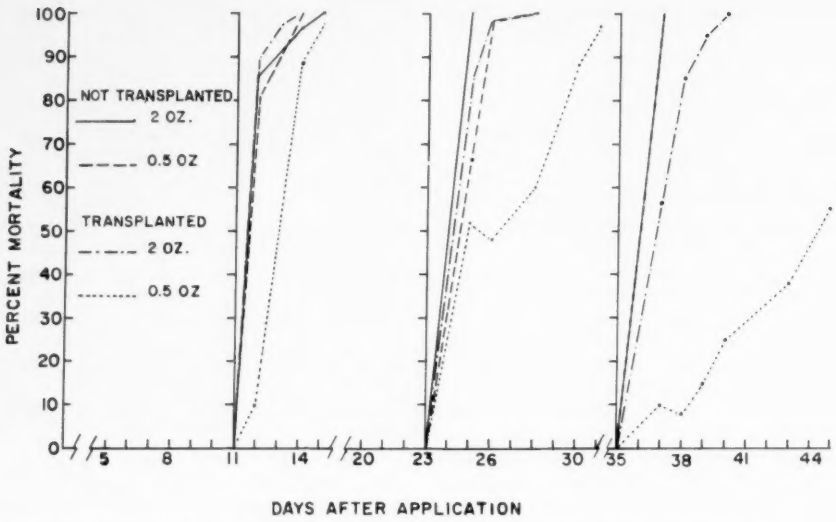

FIG. 2.—Mortality of melon aphids on chrysanthemum foliage following soil application of phorate.

Mite Tests.—Residues of phorate which were extremely toxic to melon aphids did not show the same degree of toxicity to two-spotted spider mites. In an evaluation of toxicity of phorate residues to mites, bean leaves infested with mites were placed on 4 leaves of each of 6 chrysanthemum plants per treatment. One leaf was removed from each of 3 plants per treatment on each of 7 sampling dates. The numbers of active forms, nymphs and adults, were counted in the manner previously described. Some toxicity to mites was evident following the 2-oz. application, but mites began to increase in number about 44 days after the plants were treated. (In this and other experiments, phorate residues sufficient to kill mites accumulated slowly, and never produced residues necessary for 100% control of two-spotted mites following application of rates between 0.5 and 2 oz.) Plants removed to phorate-free soil after 24 hours' exposure did not accumulate residue toxic to mites.

Biochemical Analyses.—Experiment 3. Measurements of cholinesterase inhibition were useful in identifying sites of differential accumulation of phorate residue toxins which occurred among various plant parts. In treated chrysanthemums anticholinesterase activity was found to be slight in the growing tips of immature plants. In more mature plants activity was slight in the inflorescence buds or in the open blooms. In an exploratory experiment in which plants were treated with 4, 8, or 12 oz. actual phorate, a maximum of only 114 p.p.m. phorate equivalent (measured by the method of Hensel and Curry) was found in the flowers a week following the 12-oz. application, while in the leaves of the same plants there was a maximum of approximately 1,340 p.p.m. In a similar experiment, no cholinesterase inhibition was detected in samples of inflorescences 56 days following the application of the 2-oz. rate, and only 60 p.p.m. phorate equivalent were present in the foliage of the plants that received 4 oz. of phorate. Both melon aphids and two-spotted mites confined to the inflorescences of these treated plants

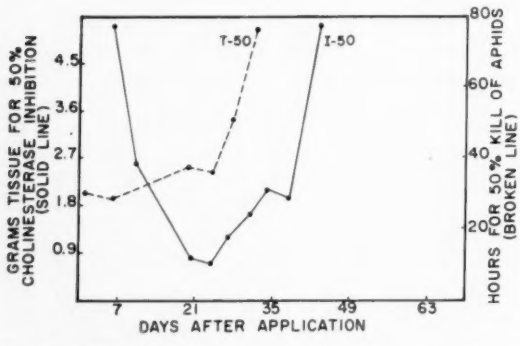
GRAMS TISSUE FOR 50% CHOLINESTERASE INHIBITION (SOLID LINE)
HOURS FOR 50% KILL OF APHIDS (BROKEN LINE)
T-50
I-50
DAYS AFTER APPLICATION

FIG. 3.—ChE inhibition by chrysanthemum foliage extracts and mortality of aphids following soil application of 0.25 ounces actual phorate per 100 gallons at 200 ml. per 6-inch pot.

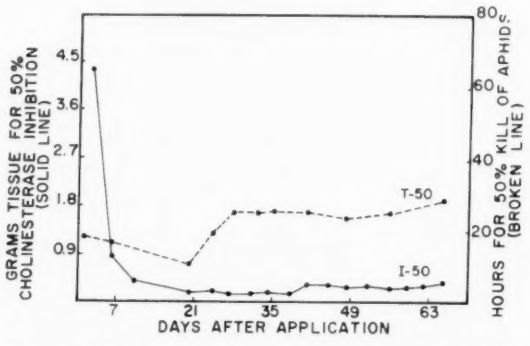
GRAMS TISSUE FOR 50% CHOLINESTERASE INHIBITION (SOLID LINE)
HOURS FOR 50% KILL OF APHIDS (BROKEN LINE)
T-50
I-50
DAYS AFTER APPLICATION

FIG. 4.—ChE inhibition by chrysanthemum foliage extracts and mortality of aphids following soil application of 2.0 ounces actual phorate per 100 gallons at 200 ml. per 6-inch pot.

survived and increased in numbers equal to those on untreated check plants.

Since sharp differences in localization of residues occurred between the flower and pooled foliage, we wished to learn if differential localization occurred among leaves along the stem. This information would have some bearing upon expected aphid and mite control and upon patterns of phytotoxicity.

Comparable electrometric analyses of the terminal 15 leaves of treated plants and the basal 9 leaves (omitting the central 8 or 9 leaves) indicated that residues following the 4-oz. application were slightly greater in the basal leaves. However, in chrysanthemums infested with leaf-curl plum aphids, *Aphis helichrysi* Kalt., it was noted that aphids survived only on the small terminal leaves and stems of the upper 1 inch of plants treated with 2 oz. actual phorate per 100 gallons. The aphid distribution was more uniform on untreated plants. Samples were taken for analysis of these terminals and of leaves below this area just prior to flower bud formation. Anticholinesterase activity of residues was found to be less in the terminal leaves which on the treated plants supported aphid infestations. In the electrometric analyses, 4.25 g. of tissue, from the terminal 1 inch of the plants were needed for 50% inhibition, whereas only 0.38 g. were needed from leaves on the lower part of the stem. Either phorate and /or its residues were swiftly metabolized to inactive residues, or translocation into terminal leaves in amounts lethal to aphids was lacking.

Cholinesterase inhibiting residues which were present following 2- and 4-oz. applications still persisted in chrysanthemum foliage at levels of activity equivalent to 245 and 1,000 p.p.m. phorate, respectively, on the 141st day after the application. Rates of 4 oz. or higher caused burning of the leaf margins which appeared about 21 days following the soil-drench treatment. The 2-oz. application controlled aphids, but did not control mites.

These results aroused our interest in the persistence pattern of anticholinesterase residues which might accrue following one brief exposure of roots to low rates of phorate. In the succeeding experiments, one-half of the plants were transplanted to phorate-free soil after 1 day's exposure to a soil drench. The activity of residues in the transplanted plants exceeded 1,150 p.p.m. phorate equivalent, figure 5, 28 days after the application of 2 oz. of phorate per 100 gallons; at 0.5 oz., 371 p.p.m. remained at 58 days.

Since the plants were exposed to phorate for only 24 hours, it was postulated that the quantity absorbed, along with the more cholinesterase inhibitory products of oxidation, accounted for the ascending portion of the curves, figure 5. The declining activity suggests the rate of hydrolytic inactivation of the final oxidative metabolites in accordance with the pattern of degradation reported by Bowman & Casida (1957). On the other hand, plants left undisturbed after the phorate application absorbed phorate or its metabolites from the soil for an extended period counteracting the residues lost by hydrolysis within the plants, figure 5.

Experiment 4. During the initial period of some 20 days following the application of phorate to soil in which chrysanthemums were grown, the number of grams of leaf tissue required for 50% inhibition of the cholinesterase in 5 ml. human blood plasma declined. A sharp drop took place during the first 7 days, figures 3 and 4.

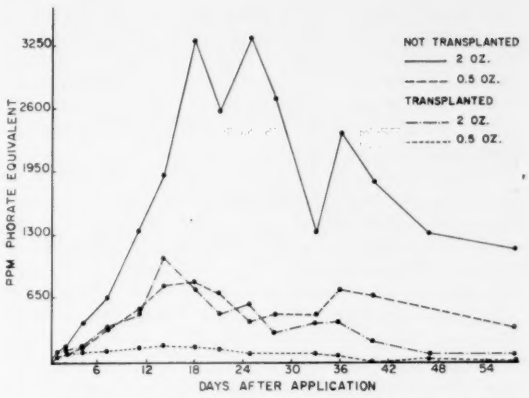

FIG. 5.—Phorate residues in chrysanthemum foliage following soil application of phorate.

Thereafter in assays of foliage from plants in a soil mixture receiving the 0.25 oz. phorate rate (fig. 3), the anticholinesterase activity continued to increase and I_{50} values reached their lowest values shortly after 21 days. From this time onward anticholinesterase activity gradually declined, more tissue was required for 50% inhibition and I_{50} values increased steadily.

The foliage of chrysanthemums growing in soil receiving the 2-oz. phorate application contained highly active cholinesterase inhibitors which persisted for more than 63 days, figure 4.

Although a high correlation between the toxicity to insects of certain organophosphorous compounds and *in vitro* inhibition of cholinesterase has been demonstrated (Chadwick & Hill 1947), it is apparent that there cannot be biologically meaningful correlation between mortality of insects on living plants and anticholinesterase value measured by the electrometric method. Neither can be related causally to the progressively changing proportion of residue toxins resulting from the degredation of phorate within plants. Nevertheless, electrometric analyses concurrent with insect mortality data were helpful in interpreting apparent differences in localization of phorate residues and the relationships of time and dosage to the distribution and persistence patterns of residues.

Upon collating the I_{50} and T_{50} curves (Experiment 2, figure 3, and Experiment 4, figure 4), the divergence between them apparent during the initial 10- to 20-day period is understandable in view of Getzin's results with phorate residues from phorate-treated soils and plants. During this period the phorate source in the soil mixture is subjected to (1) oxidation to a mixture of phorate sulfoxide and sulfone, which tends to remain the major extractable fraction in soil, (2) some hydrolysis and (3) some binding to organic matter (Getzin & Chapman 1960). The initial intake by the plant probably consisted primarily of phorate *per se* which would account for the high initial toxicity to aphids (low T_{50} values) and the associated low anticholinesterase activity (high I_{50} values) of foliage extracts. Phorate *per se* absorbed by plants may presist

for a period of time before being destroyed by oxidation and hydrolysis; e.g., applied to the base of cotton plants it was detected up to 3 days after the application. After this, only the oxidative metabolites were present (Metcalf et al. 1957). Phorate *per se* was detectable (Bowman & Casida 1958) in small amounts 17 and 32 days following soil application to vegetable crops.

It was inferred that our data reflected the fact that phorate *per se* is a relatively poor inhibitor of cholinesterase as compared with the oxidative metabolites as reported by Metcalf et al. (1957) and by Bowman & Casida (1957). On the other hand, the data also reflected the fact that phorate *per se* is highly toxic to insects as compared with most compounds in the oxidative series (Bowman & Casida 1958).

As the processes of phorate degredation within the soil and within the plant progress it would be expected that some degree of stability would attain in the relationship between the rate of aphid mortality and existing phorate residues. There is evidence of this relationship in the shape of I_{50} and T_{50} curves. At the 0.25-oz. rate (fig. 3) of phorate applied in Experiment 4 a positive correlation ($r = 0.995$) of I_{50} and T_{50} values after 20 days was significant at 0.01 probability. At the higher rate (2 oz.) the curves each became stabilized after 28 days. T_{50} remained at a continuous level requiring 25 to 30 hours for 50% mortality from 28 days following the application until termination of the experiment at 65 days, and I_{50} remained at a continuously low level requiring only 0.3 to 0.5 grams tissue for 50% inhibition during the same period.

DISCUSSION.—The range of phorate residues usuable in controlling insect and mite pests of chrysanthemums is delimited, at the lower extremity, but the threshold of effectual toxicity to the various pests, and, at the upper extremity by phytotoxicity. A third dimension, time, measured in terms of the duration of residue activity within these extremes relates to the practical usefulness of phorate phytosystemically against aphids and mites. If both melon aphids and two-spotted mites are to be controlled, the rates should be in excess of 2 oz. actual phorate per 100 gallons. Rates as low as 0.25 oz. per 100 gallons readily killed melon aphids and rates as low as 2 oz. were toxic to some mites but not sufficient to provide mite control necessary in commercial chrysanthemum production. The usable range of a single application of phorate appeared to be restricted to the narrow range between 2 and 4 oz. actual phorate per 100 gallons applied at 200 ml. per 6-inch pot under the experimental conditions. Amounts in excess of 4 oz. caused leaf margins to burn after about 20 days. The 2-oz. rate is equivalent to 8.3 oz. actual phorate per 1,000 square feet of greenhouse bench soil 6 inches deep.

Because of the differential distribution of cholinesterase inhibitory residues within chrysanthemum plants following soil applications of phorate, aphids survived on terminals of plants, and mites survived in inflorescences of otherwise systemically toxic plants.

The obscurity of the relationships between the oxidative metabolites of phorate within systemically treated plants and the toxicity of these mixtures to insects and mites points up the inadequacy of p.p.m. values (based upon phorate *per se* or upon a selected metabolite) as a basis upon which to interpret the relationships between phorate residues (estimated by cholinesterase inhibitory action) and toxicity to any plant feeder whether it be insect or man. Residue toxin values derived by bioassay methods have the advantage of characterizing the sum effect of metabolites differing in quantity and toxicity and permit a realistic interpretation of the toxicity of pesticide residues.

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Appraisal of Insecticidal Granules in the Row Against Damage by the Northern Corn Rootworm[1]

J. W. Apple,[2] *University of Wisconsin, Madison*

Abstract

A small-plot (single row 40 ft. long) experiment was used to evaluate granular insecticidal formulations in the corn row against the northern corn rootworm, *Diabrotica longicornis* (Say). Previously weighed quantities of granules were drilled into the soil above the seed by means of a V-belt seeder. A rootworm population of 45 insects per untreated plant on late-planted (May 25–26) corn resulted in plant lodging ranging from 0.6% to 100% among the various treatments. Untreated plots had 96.6% lodging. Twenty per cent heptachlor on attapulgite granules was less effective than 5% and 10% formulations and heptachlor on 8/15 mesh particles provided less protection from lodging than formulations prepared with 15/30, 20/35 and 30/60 mesh granules. These differences were demonstrated with one-half the recommended 0.5 pound per acre rate of toxicant. Attaclay, Florex and Creek-O-Nite clays were found to be satisfactory granular carrier for heptachlor. Telodrin Insecticide® (1,3,4,5,6,7,8,8 octachloro 3a,4,7,7a-tetrahydro-4,7-methanophthalan) and phorate of 13 candidate insecticides provided a high degree of protection against the rootworm when used at 1 pound per acre. Untreated corn in this test produced 16% barren stalks while protected corn averaged 2% unproductive stalks.

At the present time, aldrin and heptachlor granular formulations in the row constitute the principal method of corn rootworm control in the Midwest. This applies to the northern species, *Diabrotica longicornis* (Say), as well as the western form, *D. virgifera* LeConte. The common recommendation involves 2.5 pounds of a 20% formulation to provide 0.5 pound actual toxicant per acre. This low application rate of an insecticidal formulation resulted from the earlier use of 20% or 25% granules in starter-fertilizer (Apple 1957). The fertilizer mixtures were prepared with insecticides on 30/60 mesh granules, and such products were initially used in the direct application of granules to the row. However, during recent years a trend to coarser granules (24/48, 20/40 and 15/30 mesh sizes) has occurred in formulations applied by way of planter-mounted granule applicators. The use of larger granule particles was motivated by manufacturing and application expedience but was independent of supporting control information on rootworms or other subterranean insects.

Apple (1960) reported 98.7% reduction in northern corn rootworm lodging from the use of aldrin or heptachlor granules in the row when the recommended 0.5 pound per acre rate of toxicant was employed in a large field experiment. When the rate of application was reduced about one-half, heptachlor continued to give excellent protection, but reduction in lodging from aldrin dropped to 75%. In each case, a 20% concentration of insecticide was used on granular clay but there is no record of particle sizes involved. This preliminary test prompted an experiment in 1960, wherein insecticide concentration and granule particle size were evaluated in terms of northern corn rootworm control. In addition, the experiment attempted to determine the relative value of three clay carriers and 16 different toxicants.

Materials and Methods.—Through the courtesy of Velsicol Chemical Corporation and Minerals and Chemicals Philipp Corporation, 12 granular formulations of heptachlor were specially prepared for this test. Ten per cent and 5% concentrations were provided on AA-RVM attapulgite granules of 30/60, 20/35, 15/30 and 8/15 mesh sizes. Twenty per cent formulations involved 46.4% attapulgite, 13.6% bentonite and 6.4% Celite 209 as specified by the Velsicol Chemical Corporation for all 20% heptachlor granular formulations. Heavy aromatic naphtha solvent was employed in the preparation of all formulations. Processing was done in 100-pound lots in a concrete mixer which has a mixing action not too dissimilar from a conventional Munson Blender. All other granular formulations were supplied by interested manufacturers.

Chemical names of the proprietary materials referred to in this paper are as follows:

Amer. Cyanamid 18133—*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate.

Bayer 29493—*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate.

Diazinon®—*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.

Di-Syston®—*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate.

Dylox®—dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate.

SD-4294—α-methylbenzyl 3-(dimethoxyphosphinyloxy)-crotonate.

SD-4360—methyl 3-(methoxyphenoxyphosphinyloxy)-crotonate

Telodrin Insecticide®—1,3,4,5,6,7,8,8 octachloro 3a,4,7,7a-tetrahydro-4,7-methanophthalan. (formerly SD 4402)

Trithion®—*S*-[(*p*-chlorophenylthio)methyl] *O,O*-diethyl phosphorodithioate.

V-C 13—*O,O*-diethyl *O*-2,4-dichlorophenyl phosphorothioate.

Granular insecticides were weighed out in the laboratory in amounts to treat 40 feet of row. For application, a sample was distributed evenly onto a V-belt seeder (fig. 1) which was used to drill the granules one-half inch deep in the center of the corn row (fig. 2). The corn itself had been planted previously at a depth of 2 inches with two kernels every 20 inches. This planting rate provided 48 plants in 24 two-plant hills for each 40-foot single-row plot. Individual treatments were replicated four times in a randomized block design. Planting and insecticide applications were made on May 25 and 26 in a field used

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[2] Professor of Entomology, Department of Entomology, University of Wisconsin, Madison.

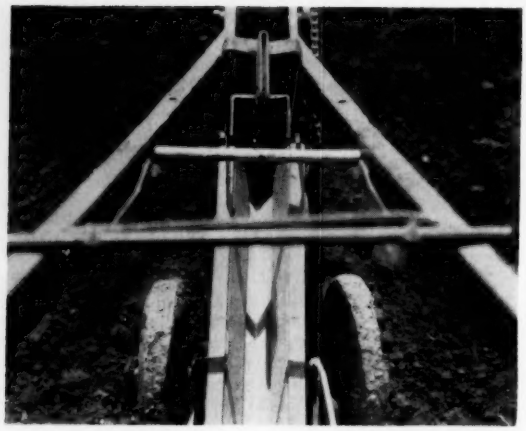

FIG. 1.—V-belt seeder used to apply granulated insecticides in the row over seeded corn.

for dent corn production during the previous 2 years.

Rootworm larvae were first detected in the fourth set of crown roots on July 5 when plants were 26 inches in extended height. Because of the late seeding date, the plants were less mature than normally encountered in early July and for this reason the subsequent root damage and lodging were more severe than commonly observed in Wisconsin. Maximum insect numbers occurred on July 28 when 36.2 larvae and prepupae, 8.5 pupae and 0.5 adults were recovered per plant. The first lodging of plants was noted July 15. Additional lodging occurred on July 25 during a 2.33-inch rain, but major plant tipping followed an August 3 storm involving 1.4 inches of rain with winds up to 36 m.p.h.

Response of rootworms to the various treatments was based on the number of lodged plants in the inner 20 hills of each plot. The average stand per plot at harvest was 39 out of 40 plants. All plants inclined from the perpendicular were considered lodged. Such a classification was overly severe but it did provide for greater spread in results than would have occurred had lodging of 30 degrees or more from vertical been employed (Hill et al. 1948). Plots receiving the better treatments had 2% to 15% of the plants showing very slight lodging while untreated plots had most of the plants reclining on the ground for more than one-half their length.

Yield data were taken in a few selected treatments and the untreated plots to show the influence of rootworms on plant yield. The ears from the 20 inner hills of a plot were harvested during November and the field weights were converted to bushels per acre of 15.5% moisture corn. Barren plants were observed and recorded during this harvest to establish if possible a relationship between rootworm lodging and failure to develop ears.

RESULTS.—Plant lodging data in the 3×4 factorial experiment involving concentrations and particle size are given in table 1. These figures were converted to angles before testing for significance by analysis of variance. Both insecticide concentration and granule size contributed to significant (5%) variation in rootworm lodging. Heptachlor at a concentration of 20% on all size

Table 1.—Northern corn rootworm lodging[a] in 3×4 factorial experiment using 0.25 pound of heptachlor per acre as granules in the row.

Mesh Size		Per Cent Plant Lodging			
		Per Cent Concentration of Heptachlor on Granules			Overall Means
		20	10	5	
30/60		7.5	15.4	0.0	
		17.5	10.0	0.0	
		7.7	5.4	0.0	
		7.7	0.0	7.5	
	Means	10.1	7.7	1.9	6.6[b]
20/35		15.0	2.7	2.6	
		12.8	7.7	2.5	
		5.0	0.0	18.4	
		5.1	5.1	2.6	
	Means	9.5	3.9	6.5	6.6
15/30		43.6	5.6	18.9	
		5.1	15.0	5.3	
		0.0	0.0	7.7	
		5.1	2.6	2.5	
	Means	13.4	5.8	8.6	9.3
8/15		52.5	17.9	26.3	
		15.8	8.1	15.0	
		48.7	25.0	0.0	
		50.0	7.7	0.0	
	Means	41.7	14.7	10.3	22.2
Overall means		18.7	8.0	6.8	

[a] Untreated plots had 96.6% lodging.
[b] Enclosed means not significantly different at 5% level of probability.

granules was less effective in protecting from rootworm lodging than insecticidal concentrations of 10% and 5%. Likewise, the coarse 8/15 mesh particles contributed less to rootworm control than the fine 15/30, 20/35 and 30/60 mesh sizes. The interaction between concentration and mesh size was not significant, although a cursory examination of the data would seem to indicate that both concentration and mesh size influenced rootworm control in relation to the other factor. Unfortunately, the within

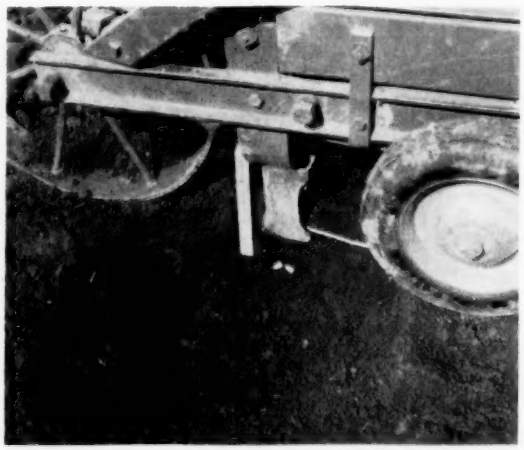

FIG. 2.—Relative position of corn seed and band of granules in the row used to control northern corn rootworm.

subclass variation was too great to secure a meaningful interaction at the 5% level of probability.

This relationship between insecticide concentration and granule mesh size was established with one-half the commonly used rate of heptachlor. Increasing the application to 0.5 pound per acre (recommended rate) or above would tend to equalize controls but in this era of judicious dissemination of pesticides every effort should be made to obtain the maximum insect protection from formulations involving the minimum effective dose.

The two attapulgite clay granules, Attaclay and Florex, proved to be equally satisfactory as a carrier for heptachlor in rootworm granules (table 2). A critical comparison at the 0.25-pound per acre rate of toxicant showed a trend to less satisfactory protection as the particles of each clay became larger. Creek-O-Nite, a montmorillonite clay, bearing 10% heptachlor was provided as a 24/48 mesh granule which was not duplicated in other heptachlor formulations. For this reason, the Creek-O-Nite comparison shown in table 2 had to be made with Attaclay granules of 30/60 and 20/35 mesh sizes. In spite of this inconsistency, it is safe to conclude that Creek-O-Nite was a satisfactory carrier under the conditions of this experiment.

The efficacy of 14 candidate insecticides against the northern corn rootworm as compared with the standard materials, aldrin and heptachlor, is given in table 3. Data are also presented on the performance of the two recommended chemicals at rates below and above the commonly used dosage of 0.5 pound per acre. In these latter comparisons no advantage was found for increasing the dosage of heptachlor and aldrin from 0.5 to 1 pound per acre. Reducing dosages to 50% of the recommended rate did not alter lodging appreciably but an 80% reduction in toxicant caused a significant increase in lodged plants in all cases except where 10% heptachlor on 30/60 mesh granules was employed. Particle counts provided by Mr. Eugene P. Ordas of the Velsicol Chemical Corporation showed that 10% heptachlor on 30/60 mesh granules provided 650 granules per foot of row in the application of 0.1 pound of heptachlor per acre while the 20% product supplied only 240 particles. This difference might explain the advantage of 10% heptachor over a 20% product at the 0.1 pound per acre comparison, but this was not true with aldrin where the 10% concentration on 24/48 mesh granules provided more particles per linear foot of row than 20% on 30/60 mesh granules. Didriksen & Lilly (1958) pointed out the importance of particle number against soil insects when they found that 1.25 pounds of heptachlor per acre provided by 394 particles of a 5% granular formulation in a pound of soil gave 73% mortality of wireworms while only 20% kill resulted from 30 particles per pound of soil derived from a 20% formulation. Apple (1961) reported 89.5% or better reduction in corn rootworm lodging from 0.25 pound of heptachlor in the row when granular particles containing 5%, 10% or 20% insecticide amounted to 274 or more per linear foot.

Of the candidate insecticides, lindane provided excellent protection from the northern corn rootworm at a rate of 0.25 pound per acre. The value of lindane against rootworms has been known for some time (Cox & Lilly 1953), but owing to its phytotoxic action on corn (Fleming & Maines 1953) it is not used as a row treatment. Telodrin showed great promise when used at 1 or 2 pounds per acre, and this chlorinated hydrocarbon is deserving of further testing at lower rates. Among the phosphate compounds, phorate and Diazinon afforded good protection from the rootworm at 1 pound per acre while Di-Syston and Trithion at 2 pounds per acre kept plant lodging at 15% or less. The remaining eight phosphate insecticides proved of no value in this experiment where it was necessary for a compound to remain toxic for at least 2 months to be effective on the larvae that began to appear about 30 days after application of insecticides.

Table 2.—Comparison of three clay carriers in granular formulations of heptachlor against northern corn rootworm.

Clay Type	Mesh Size	Per Cent Lodged Plants: Pounds Insecticide per Acre 0.1	0.25	0.50
		20% concentration on granules		
Attaclay	30/60	26.2	10.1[a]	8.2[a]
	20/35		9.5[a]	
	15/30		13.4[a]	
	8/15		41.7	
Florex	30/60	10.8[a]	7.6[a]	10.9[a]
	20/40		7.6[a]	
	16/30		12.6[a]	
	8/15		36.9	
		10% concentration on granules		
Attaclay	30/60		7.7[a]	4.4[a]
	20/35		3.9[a]	
Creek-O-Nite	24/48		2.5[a]	3.8[a]
Untreated		96.6		

[a] Mean lodging figures nonsignificant from lowest mean.

Table 3.—Evaluation of recommended and candidate insecticides in granular formulations against northern corn rootworm.

Insecticide	Per Cent Concentration	Mesh Size	Per Cent Lodged Plants: Pounds Insecticide Per Acre 0.1	0.25	0.5	1.0	2.0
Heptachlor	20	30/60	26.2	10.1[a]	8.2[a]	8.3[a]	
Aldrin	20	30/60	48.1	17.5	7.0[a]	6.9[a]	
Heptachlor	10	30/60	5.1[a]	7.7[a]	4.4[a]	3.9[a]	
Aldrin	10	24/48	55.0	10.2[a]	13.7	8.4[a]	
Lindane	5	15/30		6.5[a]	8.8[a]		
Telodrin	5	30/60				0.6[a]	4.4[a]
Phorate	10	24/48				6.5[a]	7.3[a]
Diazinon[b]	2.5	20/30				23.5	3.8[a]
Diazinon[c]	5	20/60				15.4	7.2[a]
DiSyston	10	24/48				31.2	9.1[a]
Trithion	5	20/35				54.8	15.0
B-29493	5	30/60				85.2	51.9
AC-18133	10	30/60				86.6	57.3
Ethion	5	24/48				82.9	68.7
VC-13	20	20/40				84.9	79.0
Dimethoate	10	30/60				86.4	89.3
Dylox[d]	5	24/48				92.8	88.6
Dylox[e]	5	30/60				98.1	91.6
SD-4294	5	30/60				100	91.8
SD-4360	5	30/60				95.5	100
Untreated					96.6		

[a] Mean lodging figures nonsignificant from lowest mean (5% level).
[b] On marble chips.
[c] On walnut flour granules.
[d] Impregnated on Pike's Peak clay granules.
[e] Coated on talc granules.

Table 4.—Harvest data from selected treatments applied for corn rootworm control.

INSECTICIDE (LB./ACRE)	PLANTS PER PLOT		YIELD (BU./ACRE)
	Total	Barren	
1.0 Telodrin	39.7	0.0[a]	97.4
2.0 phorate	38.2	1.0	85.2
0.1 aldrin	40.0	0.5	85.2
0.5 heptachlor	39.5	1.7	84.2
0.5 aldrin	39.7	1.2	83.5
1.0 phorate	38.5	0.7	83.5
0.1 heptachlor	39.5	0.5	82.6
2.0 Diazinon	39.2	1.2	77.6
2.0 Telodrin	39.7	0.7	74.7
Untreated	38.7	6.2	60.6
	n.s. (5%)		n.s. (5%)

[a] Barren plants per plot associated with vertical line are nonsignificant at 5% level.

Nine of the 69 treatments plus the untreated check were harvested in November to determine benefits in yield or possible reductions from the use of certain of the candidate chemicals (table 4). Although yields varied from 60.6 to 97.4 bushels per acre, there was too much unexplained variation among the various plots to obtain significance between yield means ($F=2.10$, 5% level 2.25). One reason for taking harvest data on these particular treatments was to ascertain, if possible, the influence of Telodrin, phorate and Diazinon on gross corn growth as compared with the standard treatments of aldrin and heptachlor. Phorate at 1 or 2 pounds per acre and Telodrin at 1 pound per acre did not appear to affect the corn adversely but Telodrin and Diazinon at 2 pounds to the acre resulted in yields that might possibly reflect some phytotoxicity. Plant stands at harvest did not vary significantly among the nine treatments and the check. This fact would indicate that rootworms by themselves do not necessarily reduce plant stands, even though lodging is very severe. Likewise, there is no evidence that the three experimental insecticides killed seedling plants as they grew through the narrow band of granulated chemicals. The one significant fact determined by these harvest data was the influence of rootworms and plant lodging on barrenness. Untreated plots had 16% of the plants with unproductive ears while the average of the nine treatments was only 2% barrenness. This fact in itself would account for a major loss in yield.

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A New Eriophyid Mite Infesting Bermuda Grass[1]

DONALD M. TUTTLE and GEORGE D. BUTLER, JR., *University of Arizona, Tucson*

ABSTRACT

A new eriophyid mite (*Aceria neocynodonis* Keifer) was discovered infesting a Bermuda grass lawn at Phoenix, Arizona in August 1959. Later in 1959 the new mite was also found at Yuma and Tucson causing extensive damage. Other specimens were identified from Imperial County and near Los Angeles, California. A typical rosetting and tufting of the growth caused by the shortening of the internodes occurs. Eventually lawns are thinned out where the plants are killed.

Initial chemical control measures indicated that satisfactory results can be accomplished with Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) thiophosphate) spray or dusting sulfur 325-mesh.

In August 1959 eriophyid mites were found infesting a Bermuda grass lawn at Phoenix, Arizona by Dr. J. N. Roney, Extension Entomologist of the University of Arizona. Samples were sent to Mr. H. H. Keifer, of the California Department of Agriculture, for determination. The species was found to be new and was subsequently described as *Aceria neocynodonis* by Keifer (1960). Another eriophyid mite, *Aceria cynodonis* Wilson, infests Bermuda grass in coastal southern California and in northern California, but does not range to the Imperial Valley or to Arizona, and does no apparent damage (H. H. Keifer, personal communication). Following its initial discovery this new mite was found also in 1959 in several ailing Bermuda grass lawns at Phoenix, Tucson and at Yuma, Arizona, where extensive areas of brown and dying lawns were observed. During the spring and summer of 1960 the mite occurred in Bermuda grass lawns throughout southern Arizona. V. D. Roth reported the same eriophyid mite during 1960 in Imperial County, California, occurring abundantly in lawns and on golf courses. In September 1960, H. H. Keifer identified specimens of this species collected near Los Angeles, California. It is probable that this mite has been present for some time as suggested by its widespread distribution, although the characteristic damage and the browning

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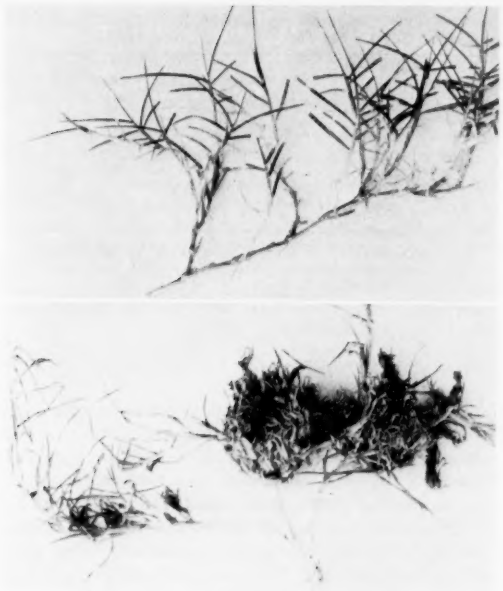

FIG. 1.—Bermuda grass: Above, normal growth; below, infested growth.

out of entire lawns had not been observed before in Arizona. Generally Bermuda grass is very persistent and does well during the high summer temperatures of southwestern desert areas.

DAMAGE.—The first noticeable damage to Bermuda grass was observed in the spring when lawns failed to begin their normal growth in spite of irrigations and applications of fertilizer. The grass that did appear was damaged by the mites and displayed a typical rosetting and tufting of the growth (fig. 1), caused by a shortening of the internodes and the apparent stimulation of abnormally excessive plant growth. The mites remained hidden under the leaf sheaths and varied in number from a few to a hundred or more under a single sheath. With heavy infestations the grass turned brown and died. Eventually the grass, in infested lawns, became greatly thinned out, allowing the growth of weeds.

There was such an array of cultural and management conditions found in the series of home lawns observed in 1960 that it was difficult to determine which were the most important factors influencing mite populations. The variety of grass was a significant factor in susceptibility to attack. Table 1 shows a rating of mite injury made in a series of replicated Bermuda grass pasture and hay study plots maintained by the Department of Agronomy, University of Arizona. Arizona Common was severely injured while several other varieties had no injury. Bermuda grass strains and varieties grown in pots under lath house conditions showed extreme variations in response to mite injury. Dr. A. A. Baltensperger of the Department of Agronomy, University of Arizona, observed that of 218 selections, 25 showed severe symptoms (score 9), 115 showed no symptoms (score 0) while others showed intermediate symptoms. A few of the varieties

Table 1.—Rating of damage by an eriophyid mite to various Bermuda grass varieties.[a] Tucson, Arizona, September 2, 1960.

VARIETY	PASTURE CUTTING	HAY CUTTING
Arizona Common	33	37
Cruz	24	21
Suwannee	20	20
Greenfield	14	15
GA 4-30	11	15
NK 37	11	12
Midland	11	10
Coastal	10	10

[a] Each plot, in five replicates and two types of cuttings, was rated from 1, for no injury, to 4, for severe injury; therefore ratings of 10 indicate no injury and of 40 for severe injury.

observed were scored as follows: Ormond 9, Tifgreen 7, Sunturf 1, Uganda 0 and Tifway 0. Selections from Common Bermuda varied from a score of 0 to 9, indicating that breeding and selection might be useful in developing mite-resistant strains. In home lawns common Bermuda was most severely injured but portions of Tiffine and Tifgreen lawns were also found to be infested.

Well-fertilized Bermuda grass was much more attractive to the mites than poorly fertilized grass. A soil fertility experiment (table 2) with closely mowed Bermuda grass was examined. All fertilized plots were heavily infested, as indicated by the typical swollen and brown stems. Unfertilized grass showed almost no injury.

Examinations of large numbers of samples of mite-damaged grass by Dr. A. M. Boyle, Department of Plant Pathology, University of Arizona, showed that summer blight fungus, *Helminthosporium* sp., was always present. It seemed probable that the actual killing of large areas in mite-infested lawns was actually caused by this fungus rather than by the feeding of the mites. The fungus was able easily to invade the chlorotic, distorted grass tissues and to progress swiftly in plants already weakened by the mites.

The mites appeared to be less abundant in lawns where flood irrigation was used. In general, infestations were most apparent on dry ridges and along the margins of the lawns. Lawns that were mowed closely showed the most injury, except for golf courses that received frequent close mowings. The shorter plants may be subjected to conditions of lower humidity when cut infrequently. In some cases the damage was more severe in shaded or partly shaded areas around the edges of the lawns. This

Table 2.—The effect of fertilization of Bermuda grass upon the injury caused by the eriophyid mite.[a] Tucson, Arizona, September 9, 1960.

	FERTILIZER	RATING
16-20-0	Ammonium phosphate-sulfate	32
16-16-8	A nitric phosphate	32
21-0-0	Ammonium sulfate	31
15-6-3	Fortified fish base liquid	31
38-0-0	Urea formaldehyde	25
2-2-2	Sewage sludge	24
None	Untreated	13

[a] Recently mowed common Bermuda grass with various fertilizer plots arranged in four blocks of randomized plots was rated by two people according to the extent of injury, as in table 1.

Table 3.—Number of mites collected on September 29 from 12 grams of hand-collected infested grass from 10′×20′ plots of common Bermuda grass treated September 16,[a] Tucson, Arizona, 1960.

INSECTICIDE (RATE PER GAL.)	NUMBER OF MITES
Diazinon 12.5%, 30 cc.	0
Dusting sulfur (325 mesh), 93%	4
Isotox®[b] 30 cc., plus calcium polysulfides 25%, 15 cc.	1,548
Duocide®[c] 30 cc.	2,559
Untreated	1,555
Untreated	2,646

[a] Spray treatments were applied with a tank-type sprayer at the rate of approximately 1 gallon per 200 square feet.
[b] Malathion 10%, lindane 5%, DDT 5%, Tedion® 3% (*p*-chlorophenyl 2,4,5,-trichlorodiphenyl sulphone).
[c] Malathion 50% plus Karathane® (dinitro(1-methylheptyl)phenyl crotonate).

might have been in part due to the stressed moisture condition of plants in these locations.

An examination of Bermuda grass seed fields at Yuma indicated the prevalence of the mite around the edges and along irrigation ditches of some fields. Occasionally, it could be found in fields where the stand was sparse.

CONTROL.—A number of acaricides, used alone and in combinations, were evaluated for their effectiveness in controlling the eriophyid mites. Sprays were applied with a 3-gallon tank-type sprayer. Sulfur dust was applied with a knapsack puff duster using approximately 1 pound per 100 square feet. Granular formulations were applied with a lawn spreader at the rate of 1 pound of actual toxicant per 1,000 square feet.

Table 4.—Number of mites on September 16 taken from 16 grams of common Bermuda grass collected with a lawn mower from two replicates of 3′×6′ plots sprayed September 7.[a] Tucson, Arizona, 1960.

INSECTICIDE (RATE PER GAL.)	NUMBER OF MITES
Isotox, 20 cc., plus calcium polysulfides 26%, 15 cc.	2
Sulfur Dust (325 mesh) 93%	13
Duocide, 30 cc.	16
Diazinon 12.5%, 30 cc.	18
Malathion 57%, 30 cc.	226
Ortho Experimental Lawn Spray®[b] 30 cc.	531
Tedion WP 25%, 2 tablespoons	1,195
Untreated (1)	730
Untreated (2)	2,136

[a] A wetting agent, Triton X-100, was added at the rate of 8 cc. per gallon. Plots were sprayed with a tank-type sprayer at the rate of approximately 1.4 gallons per 100 square feet.
[b] Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) 16%, toxaphene 20%, lindane 4%.

Table 5.—Number of eriophyid mites collected from 30 grams of Bermuda grass 3 and 10 days after treatments were applied on September 13.[a] Yuma, Arizona, 1960.

INSECTICIDE	NUMBER OF MITES AFTER TREATMENT	
	3 days	10 days
Dimethoate 8%	32	12
Parathion 5%	141	212
Di-Syston®[b]	550	231
Untreated	2,160	1,940

[a] The applications were made with a lawn spreader at the rate of 1 pound of actual toxicant per 1,000 square feet. Samples were taken at random and placed in a Berlese trap for 12 hours.
[b] *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate.

Samples of Bermuda grass were collected after treatment either by hand cutting heavily infested plants or by cutting the grass very close with a mower. Four-gram subsamples were put into pint ice cream cartons and a circular piece of screen wire placed in the opening to hold the grass as the carton was inverted over a close-fitting petri dish. Cartons were placed on a damp towel with a radiant heater above. The mites dropped on the petri dishes and were counted after approximately 24 hours.

The results of using sprays and dusts are shown in tables 3 and 4. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) spray containing 30 cc. of 12.5% Diazinon in a gallon of water applied to 200 square feet and sulfur dust at the rate of 1 pound per 100 square feet gave the best control. Combinations of malathion with other insecticides and/or fungicides gave inferior results at the rate of 1 gallon per 200 square feet. When a wetting agent was added and the sprays were applied at a rate of 2.8 gallons per 200 square feet these formulations gave good control. Malathion alone at the rate of 30 cc. per gallon per 200 square feet gave poor control, as shown by observations in a number of home yards.

Table 5 presents the results of control experiments evaluating granulated insecticides. Dimethoate was the most effective treatment. The plots were watered immediately following application of the granules and it is possible that there might be a variation in the results obtained if irrigation were delayed.

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A Physiological Response of Potato to Foliar Applications of the Insecticide, Guthion[1]

J. T. Schulz, *North Dakota Agricultural Experiment Station, Fargo*

Abstract

Use of the phosphate triazine insecticide, Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate) as a foliar spray for potato insect control has resulted in increased yields of red-skinned potatoes above that normally obtained as a result of efficient insect and disease control. The magnitude of this increase averaged about 15% and was solely attributable to increases in set of tubers. Dry matter and potato grade based on size was not affected. While tuber set of white-skinned potatoes was also increased slightly, results were not significant, and yields were less than those obtained from other plots treated with insecticides.

Control of crop-infesting insects following the use of the organic phosphate insecticide, Guthion® (*O,O*-diemthyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate), has been reported by several workers. Publications by Cowan and co-workers (1958), Getzin (1960), Hacskaylo & Scales (1959) and Peay (1959) have indicated that while a diversity of crops has been subjected to applications of this insecticide, physiological effects manifested by the plant following such applications have been recorded only on cotton. Hacskaylo and Scales observed that cotton plants treated with Guthion, 0.25 pound per acre, produced more flowers than untreated plants; also a slight increase in bolls set and the delaying of plant maturation were reported. Published reports were not found which indicated similar effects on vegetable crops following applications of Guthion. The results of work reported herein show that the use of Guthion on potato, *Solanum tuberosum* L., during the growing seasons of 1958–1960 resulted in increased potato yields above that normally found to have been the result of efficient insect and disease control.

Methods.—A. 1958.—Norland, a red-skinned potato, was planted in an experiment (St. Thomas, N. Dak.) designed primarily for evaluation of the performance of candidate insecticides in the control of potato-infesting insects. Guthion and other chemicals evaluated were assigned at random within each of the four replicates in a randomized block design; within a replicate, each insecticide was applied to eight 50-foot rows with a conventional row-crop sprayer. Three applications of insecticides were made. Yield data were obtained by harvesting the four center rows of each plot. Since no attempt was made to grade and weigh the tubers in each size class, yields represent the combined weight of Grade A- and B-size tubers.

B. 1959.—The red-skinned potato, Red Pontiac, was planted at Voss, N. Dak.; replication and randomization of treatments and methods of application of insecticides were carried out as indicated previously, with five applications being made. Yields were determined by the method previously outlined.

C. 1960.—1.—Insecticide performance plots similar to those of 1958 and 1959 were used in 1960, and Guthion was included for evaluation. At Voss, a white-skinned potato, Kennebec, was planted; at St. Thomas, Red Pontiac was utilized. Replication, randomization of treatments, methods of application of insecticides and of harvesting plots were carried out in the same manner as previously indicated. Five applications at Voss and four at St. Thomas were made during the season. Plots were 100 feet long and four rows wide. Yield data were taken from the two center rows.

2.—A more detailed field study was carried out in a plot established at the Red River Valley Potato Research Farm, Grand Forks, N. Dak. Two varieties of potato, Red Pontiac and Cobbler (white-skinned), were each planted in four 50-foot rows per plot with the plots replicated four times in a randomized block design. Guthion, 0.25, 0.5 and 1.0 pound active ingredient per acre, was applied five times at 2-week intervals by use of a knapsack sprayer, the first treatment being made on June 17. A recommended insecticide, endrin, 6 ounces actual per acre, was also included as a standard for comparison. On August 29, twenty-five hills per treatment in each replicate were harvested individually and all data recorded on a per hill basis. Information from each hill sample included:

(a) Total weight of tubers in a hill.
(b) Number of tubers per hill.
(c) Number of tubers in each hill grading A or B for size.

Specific gravity on seven samples of tubers from each insecticide treatment was also determined. An average specific gravity of the seven samples was derived.

Results.—Potato yields obtained in 1958, a year of excessive rainfall and low population levels of potato-infesting insects (aphids and leafhoppers almost nonexistant; flea beetles of economic importance in July only), were generally lower than that normally obtained in years of more optimum climatic conditions. Yields from plots receiving applications of Guthion (table 1) averaged about 10% higher than those obtained from other treated

Table 1.—Yields of Norland and Red Pontiac potatoes at St. Thomas and Voss, North Dakota, 1958–1959.

Insecticide (lb. toxicant/a.)	1958 Norland (bu./A.)[a]	1959 Red Pontiac
Guthion, 0.5	280.6	370.4
Thiodan,[b] 0.5	264.9	336.7
Endrin, 0.25	258.1	—
0.375	—	310.2
Untreated	255.0	309.3
Phorate, 3	254.8	314.2

[a] Duncan range test; differences not significantly different at the 5% level.
[b] Thiodan® = 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide.

[1] Technical paper No. 11, North Dakota Agricultural Experiment Station, Fargo, North Dakota. Accepted for publication January 31, 1961.

Table 2.—Yields of Red Pontiac and Kennebec potatoes, St. Thomas and Voss, North Dakota, 1960.

INSECTICIDE (LB. TOXICANT/A.)	RED PONTIAC (BU./A)[a]	KENNEBEC (BU./A.)
Phorate, 3	—	425.9
Guthion, 0.5	477.7	308.7
Thiodan, 0.5	346.3	297.5
0.75	439.5	—
Endrin, 0.375	374.6	—
Untreated	287.0	222.9

[a] Duncan range test; differences not significantly different at 5% level.

plots. In 1959, a year of high insect abundance (leafhoppers and flea beetles of economic importance from late June to mid-August), yields from Guthion-treated plots were significantly higher, about 10%, than those from plots receiving other treatments, despite the fact that the schedule of applications under which Guthion was applied did not give commercial insect control.

Results obtained in 1958 and 1959 with red-skinned potato varieties were responsible for the increased emphasis in 1960 for an explanation of the factors responsible for the differences in yields obtained. Within the insecticide evaluation plots established in 1960, significant increases in potato yields were again recorded in plots which had received applications of Guthion. As shown in table 2 this stimulation was manifested only in plots of the red-skinned variety, Pontiac, since similar applications to Kennebec variety plots failed to result in correspondingly high yields. At no time was there any discernible difference in plant growth resulting from the application of this insecticide.

Table 3.—Yields and grading information on Red Pontiac and Cobbler potatoes treated with Guthion and endrin. Grand Forks, North Dakota, 1960.

INSECTICIDE (LB. TOXICANT/A.)	YIELD (LB./100 HILLS)	TUBERS/ 100 HILLS	GRADE A (%)	AVERAGE SPECIFIC GRAVITY
A. Red Pontiac				
Guthion, 0.25	107.0	505	64.7	1.085
0.5	126.1	566	70.3	1.089
1.0	114.3	514	68.5	1.085
Endrin, 0.375	105.8	463	71.0	1.087
Untreated	95.5	430	71.2	1.087
B. Cobbler				
Guthion, 0.25	105.7	429	72.0	1.090
0.5	121.1	499	73.1	1.090
1.0	113.2	471	71.4	1.091
Endrin, 0.375	126.4	458	77.4	1.089
Untreated	91.8	378	68.1	1.091

The primary reason for increases in yield within plots of red-skinned potatoes receiving applications of Guthion was the effect on the set of tubers. Data given in table 3,A indicate that the increased yields were almost solely attributable to the increased set. The magnitude of the increases in yield in plots which had received Guthion treatments ranged between 10% and 25% when compared with untreated and endrin-treated plots. Results obtained in this controlled study closely confirmed results obtained from plots which were handled more in accordance with commercial operations during the 3-year period of these observations.

While the optimum effect was produced following the use of the 0.5-pound rate of Guthion, the 0.25- and the 1.0-pound rates also resulted in significant increases in set of tubers. The 0.25-pound rate was not considered satisfactory for commercial insect control.

No apparent influence on tuber size or on dry matter was evident, since both treated and untreated potatoes graded 70% A tubers for size, and the specific gravity was not affected. While there was a slight increase in set of tubers within the Cobbler plots (table 3,B) significant differences were not obtained. Reasons for the differences obtained between white and red-skinned varieties cannot be explained since little is known about possible physiological differences that may exist between red and white varieties.

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Specificity of Bioassay of Insecticide Residues, with Special Reference to Phosdrin[1]

YUN-PEI SUN and JOHN SANJEAN[2]
Agricultural Research Division, Shell Development Company, Modesto, California

ABSTRACT

The specificity of bioassay methods can be greatly improved by means of physical and chemical cleanup, differences in physiological response of insects, or a combination of these factors. Characteristic properties of several common insecticides have been presented. Based upon high insect toxicity, high speed of action and low masking effect of plant extractives, a relatively specific bioassay method has been developed for assaying Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) residues in the presence of any one or a mixture of two to four of 11 cholinesterase-inhibiting insecticides. One p.p.m. of Phosdrin can be detected in several crops without cleanup, and 0.1 p.p.m. with cleanup. Based on the alkali stability, clay-catalyzed decomposition, chromatographic properties and differential toxicity to insects, schemes for specific bioassay of aldrin, dieldrin, or endrin in mixed residues are suggested.

The widespread use of many synthetic organic insecticides and the requirement for low tolerance of highly toxic materials on food crops have increased the urgency of developing sensitive, specific methods for determining microquantities of insecticide residues. Many methods have been tried in recent years. Among them, ultraviolet, infrared, gas chromatographic, radiochemical, colorimetric, enzymatic, total chloride and bioassay methods have been more extensively studied either for research purposes or for routine analyses. Each method has its advantages and limitations.

Although colorimetric methods of analyses are considered as specific and are widely applied, each method varies in the degree of specificity. Gunther & Blinn (1955) repeatedly stressed that few, if any, analytical methods are absolutely specific for a single compound. In the procedure for analyzing each pesticide they listed the interferences for each method. For example, parathion is determined via chemical treatment of its aromatic nitro group; any aromatic nitro or amino group will interfere unless removed before the reduction step. Therefore, without a proper separation, para-oxon, methyl parathion, and other similar analogs of parathion would be analyzed as parathion. In addition, plant or animal extractives may also give interferences by producing turbidity or interfering colors. Thus, it clearly indicates that the degree of specificity of so-called specific methods depends largely upon proper cleanup.

On the other hand, bioassay is generally considered as nonspecific, because any toxic materials could kill test organisms. Based upon several years of experience on the bioassay of several insecticides in a great variety of plant and animal tissues, their extractives are generally not toxic, especially after cleanup, to the house fly, *Musca domestica* L., or vinegar fly, *Drosophila melanogaster* Meig., when tested by the "dry-film" method (Sun & Sun 1952). Therefore, the principal difficulty of searching for a specific bioassay method is to distinguish one toxicant from another. This may be achieved by physical or chemical separation or by differences in insect toxicity or insect reaction to different toxicants.

Occasional attempts have been made in this and other laboratories to bioassay one insecticide residue in the presence of another. Laug (1948) found that the toxic response of the house fly to the *gamma* isomer of BHC was characteristic, because 100 to 200 times the amount of *gamma* isomer was required for the *alpha*, *beta*, *delta* and *epsilon* isomers of BHC to give the same kill of house flies. Based on the difference in the volatility and speed of action of chlordane and DDT, Fleming *et al.* (1951) bioassayed the mixture in soil extracts. Klein *et al.* (1956) tried to prove the presence of endrin residues in leafy vegetables by using alkali treatment, because endrin is stable in alkali while others, except aldrin, dieldrin and isodrin, are unstable by the same treatment. Sun (1957) mentioned that by alkali treatment, aldrin or dieldrin residues were bioassayed in his laboratory in the presence of DDT or parathion. He also suggested a combination of physical, chemical and biological means for the bioassay of a specific residue in a mixture. However, no serious studies have been made previously to determine a specific residue in the presence of one or more of a given group of insecticide residues having similar or different properties.

This report covers the findings on the separation and detection of certain insecticide residues in relation to some of their physical and chemical properties, as well as their reactions to insects.

BIOASSAY PROCEDURE.—There are no large differences in the procedures for a specific and a nonspecific bioassay method. The principle of either method is to compare the mortalities of insects obtained from exposure to a portion of treated extract to those from a series of standards, when tested under the same conditions (Sun & Sun 1952).

In the case of specific bioassay of Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) residues, the procedure described by Sanjean *et al.* (1960) has been used and summarized as follows:

To prepare a standard curve, 2 mg. (2 ml. of 0.1% solution) of peanut oil, 2 ml. of an untreated check extract, and measured volumes of insecticide standard solution are added to each 4-oz. wide-mouth jar which is then placed on a mechanical shaker[3] in a ventilated hood to evaporate the solvents. For treated samples, only peanut oil and treated extract are used. About 5 minutes after the solution is evaporated to dryness, 50 CO_2-anesthetized unsexed 1-day-old vinegar flies, *D. melanogaster*, are counted into a small glass cup and transferred into each jar. A piece of cotton soaked with apple juice is placed in the jar which is then covered with a piece of

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[2] Present address: P. O. Box 242, Franklin Park, Illinois.

[3] Catalog No. 8917–A, Arthur H. Thomas Co., Philadelphia, Pennsylvania.

tissue paper. Mortality counts are made at various time intervals (3 to 24 hours). Both dead and moribund flies are counted as dead. Unless otherwise mentioned, *Drosophila* were used in the following studies.

From the average mortality of flies, the amount of insecticide residue (μg.) in a treated sample is read directly from the dosage-mortality curve of the standard. This is then converted to p.p.m. by the following equation:

$$\text{Residue in treated sample, p.p.m.} = \frac{\text{Apparent toxicant found (μg./jar)}}{\text{Extraction ratio (g./ml.)} \times \text{Vol. of extract per jar (ml.)}}$$

CHARACTERISTIC PROPERTIES OF INSECTICIDES.—It has been mentioned that in bioassay, interference is mainly due to the toxicity of other insecticides. Therefore, in order to find a more specific bioassay method, characteristic properties of insecticides, especially the one under investigation, should be thoroughly investigated so that possible clues may be found to reduce the interference of possible contaminating toxicants. Since under normal conditions of application insecticide residues on plants are usually low, less than 10% interference from any one contaminant is arbitrarily permissible for a specific method.

In the following studies on seven characteristic properties, Phosdrin was selected as an example for most of the cases. If one property cannot reduce the interference to the required level, a combination of two or more properties may be used.

Toxicity to Resistant Insects.—As is known, insects resistant to chlorinated hydrocarbon insecticides have little or no cross resistance to organophosphorus compounds. Therefore, organophosphorus insecticide residues can easily be distinguished from those of chlorinated hydrocarbons by exposing susceptible and resistant strains of insects to the "dry-film" residues (Sun & Sun 1952) or to the macerated tissues (Pankaskie & Sun 1952) by the differences in mortality and in the slope of the dosage-mortality curves. This property can be used to essentially eliminate interferences from chlorinated hydrocarbon insecticides in a bioassay for an organophosphorus compound as in the following example for Phosdrin and dieldrin.

Four-day-old susceptible (NAIDM (National Association of Insecticide and Disinfectant Manufacturers) 1948 strain) and resistant house flies (resistant to chlorinated hydrocarbon insecticides) were exposed to various amounts of dieldrin and Phosdrin in 8-oz. jars each containing 1 ml. of celery extract (equivalent to 0.5 g. of sample) and 1 mg. of mineral oil. Mortality counts were made 24 hours later. The LC_{50}'s for the susceptible strain are 0.6 and 2.8 μg. per jar for Phosdrin and dieldrin respectively and those for the resistant strain are 1.2 μg. per jar for Phosdrin and >1,000 μg. per jar for dieldrin. The degree of interference of dieldrin is:

$$\frac{0.6}{2.8} \times 100$$

or 21.4% when tested with the susceptible flies and less than 0.12% with the resistant flies.

Insect Toxicity.—Insect resistance to certain insecticides or classes of insecticide is one form of selective toxicity. There are other characteristic properties which might be useful to develop a specific bioassay method. Among them, the selective or differential toxicity of a compound to a certain species of insect may be useful. Since Phosdrin is highly toxic to many species of insects, there seems to be a good possibility of finding one species more susceptible to Phosdrin than to other insecticides. Among the species of insects tested, it was found that *Drosophila* are highly susceptible to Phosdrin. The LC_{50}'s (table 1) obtained by the bioassay method described above indicate that, for the time intervals investigated, Phosdrin is the most toxic of 12 insecticides tested. When 24-hour mortality counts are used for comparison the interference is rather high (10.5% to 51%) for methyl parathion, malathion, para-oxon, parathion, Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate) and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate, but low (<0.1% to 8.7%) for others.

Speed of Action.—In laboratory and field tests Phosdrin has shown high speed of action. If the mortality reading is taken a few hours after treatment, the toxic action of Phosdrin should show up much earlier than others. With this property in mind, the following experiment was designed as an additional means for separating Phosdrin from 11 cholinesterase-inhibiting insecticides.

To compare the speed of action, the LC_{50}'s for Phosdrin, parathion, malathion, para-oxon, Diazinon, methyl para-oxon, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Guthion, demeton, TEPP, ethion, and Sevin® (1-naphthyl *N*-methylcarbamate) were determined by the above bioassay method in the absence of plant extract at 3-, 4-, 5- and 24-hour exposures. Of the compounds tested, Phosdrin is not only the most toxic to vinegar flies but is also faster in action, as indicated by general decreases in interference at shorter time intervals. At 3- and/or 4-hour exposures, all, except Diazinon and TEPP, give less than 10% interference (table 1).

The speed of action for each toxicant is further characterized by the R_t value, the ratio of LC_{50}'s between two time intervals (table 1).

$$R_t = \frac{LC_{50} \text{ at x hour}}{LC_{50} \text{ at y hour}}$$

In this equation, x may be 3-, 4-, or 5-hour exposure and y may be 24-hour or even longer exposure. The R_t values in combination with the insect toxicity could give more specific identification of Phosdrin residues. For example, para-oxon and Diazinon give higher interference, but their R_t values (3 hr./24 hr.) are 14.5 and 8.3, respectively, as compared with 2.7 for Phosdrin. The average of five determinations of R_t value (4/24 hr.) for Phosdrin is 2.1 ±0.093 (S.E.), indicating a satisfactory reproducibility. Based on the LC_{50}'s and R_t values, it is obvious that shorter exposure (3 hr.) favors the differentiation between Phosdrin and other residues and that longer exposure (4 or 5 hr.) gives better sensitivity. To compromise these two effects, 4-hour exposure has been used for most of the studies. Other time intervals may also be used where a

Table 1.—LC_{50} and R_t values of several cholinesterase-inhibiting insecticides and their interference on the bioassay of Phosdrin residues without plant extract and with celery extract.

Series	Toxicant	LC_{50}, μg./Jar at				Interference (at LC_{50}) as Apparent Phosdrin, %				R_t Values		
		3 Hr.	4 Hr.	5 Hr.	24 Hr.	3 Hr.	4 Hr.	5 Hr.	24 Hr.	3/24 Hr.	4/24 Hr.	5/24 Hr.
					A. Without plant extract							
I	Phosdrin	1.2	0.92	0.67	0.44	—	—	—	—	2.7	2.1	1.5
	Parathion	24	10	4.6	1.9	5.0	9.2	14.6	23.2	12.6	5.3	2.4
	Malathion	26	14	7.5	2.5	4.6	6.6	8.9	17.6	10.0	5.6	3.0
	Para-oxon	29	7.8	6.0	2.0	4.1	11.8	11.2	22.0	14.5	3.9	3.0
	Diazinon	7.1	4.4	3.6	0.86	16.9	20.9	18.6	51	8.3	5.1	4.2
	Methyl para-oxon	28	15.5	11	4.2	4.3	5.9	6.1	10.5	6.7	3.7	2.6
II	Phosdrin	1.2	0.91	0.77	0.33	—	—	—	—	3.6	2.8	2.3
	Trithion	310	100	45	12.4	0.40	0.90	1.7	2.7	25	8.1	3.6
	Guthion	100	28	10	1.0	1.2	3.2	7.7	33	100	28	10.0
	Demeton	47	33	30	11.4	2.6	2.8	2.6	2.9	4.1	2.9	2.6
	TEPP	9.0	7.6	5.7	3.8	13.3	12.0	13.5	8.7	2.4	2.0	1.5
	Ethion	156	55	55	19	0.8	1.7	1.4	1.7	8.2	2.9	2.9
	Sevin	>400	>400	>400	>400	<0.3	<0.2	<0.2	<0.1	—	—	—
					B. With celery extract							
I	Phosdrin	0.73	0.49	0.40	0.224	—	—	—	—	3.3	2.2	1.8
	Parathion	23	11.5	7.3	4.4	3.1	4.3	5.5	5.2	5.2	2.6	1.7
	Malathion	21.5	13.8	11.2	8.8	3.4	3.5	3.6	2.5	2.4	1.6	1.3
	Para-oxon	16.7	11.5	8.2	5.4	4.4	4.3	4.9	4.1	3.1	2.1	1.5
	Diazinon	9.6	7.5	6.3	4.0	7.6	6.5	6.3	5.6	2.4	1.9	1.6
	Methyl para-oxon	9.2	6.2	5.5	3.8	7.9	7.9	7.3	5.9	2.4	1.6	1.4
II	Phosdrin	0.79	0.62	0.48	0.28	—	—	—	—	2.8	2.2	1.7
	Trithion	640	140	64	13	0.12	0.44	0.75	2.2	49	11	4.9
	Guthion	45	38	32	17	1.8	1.6	1.5	1.6	2.6	2.2	1.9
	Demeton	340	170	140	71	0.23	0.36	0.34	0.39	4.8	2.4	2.0
	TEPP	73	68	68	61	1.1	0.91	0.71	0.46	1.2	1.1	1.1
	Ethion	290	160	100	80	0.27	0.39	0.48	0.35	3.6	2.0	1.3
	Sevin	200	210	240	240	0.40	0.30	0.20	0.12	0.83	0.87	1.0

specific advantage is needed.

Masking Effect.—It is a well-known fact that plant extractives, if not toxic, generally mask in varying degree the toxicity of insecticides (table 1A and B). This masking effect may be expressed as follows:

$$\text{Masking ratio} = \frac{LC_{50} \text{ of Compound A with extractives}}{LC_{50} \text{ of Compound A without extractives}}$$

Since the necessary data for this group of compounds have been obtained in table 1A and B, the masking ratio can be calculated by the above equation. The higher masking ratio indicates the greater masking of toxicity. Results in table 2 indicate that with celery extracts some of them, such as Phosdrin, have little change in masking ratio at different exposure time intervals, while others have moderate to high changes in masking ratio. The greatest change of masking ratio occurs to Guthion, varying from 0.45 at 3-hour exposure to 17.0 at 24-hour exposure. These values offer additional clues in selecting test conditions for the specific bioassay of one toxicant in the presence of one or more of the others.

Partition Distribution.—In chemical analysis the separation of insecticides from interfering extractives is often performed by partition distribution. This is done with two immiscible liquids, with the insecticide more soluble in one and the interfering extractives more soluble in the other. Gunther & Blinn (1955) have extensively reviewed this technique in relation to residue analysis.

Since the interferences of toxicants is a critical factor in bioassay, the partition technique can be used for the separation of interfering toxicants. For example, Phosdrin is water miscible and most insecticides are generally slightly soluble or insoluble in water. The liquid-liquid partition technique offers a great opportunity for the separation of Phosdrin from others. Using the *n*-hexane-water partition, the interference of Diazinon has been reduced from 6.9% to 1.0% in specific bioassay of Phosdrin in celery extracts (Sanjean *et al.* 1960).

Column Chromatography.—Column chromatography has been generally used for removing interfering extractives in residue analysis. This technique involves the

Table 2.—Masking effect of celery extracts on several cholinesterase-inhibiting insecticides.

Toxicant	Masking Ratio at the Following Exposure			
	3 Hr.	4 Hr.	5 Hr.	24 Hr.
Phosdrin	0.63	0.60	0.61	0.65
Parathion	0.96	1.2	1.6	2.3
Malathion	0.81	0.99	1.5	3.5
Para-oxon	0.58	1.5	1.4	2.7
Diazinon	1.4	1.7	1.7	4.6
Methyl para-oxon	0.33	0.40	0.50	0.90
Trithion	2.1	1.4	1.4	1.0
Guthion	0.45	1.4	3.2	17.0
Demeton	7.2	5.2	4.6	6.2
TEPP	8.1	8.9	11.9	16.1
Ethion	1.9	2.9	1.8	4.2

selection of proper solvents and solid adsorbents, so that the toxicant would pass through the column in solution while the interfering extractives selectively adsorb on the column. In bioassay other insecticides usually give a certain amount of toxicity which cannot be distinguished from that of the toxicant to be bioassayed. Column chromatography gives an additional means to separate these interfering insecticides.

For example, in the specific bioassay of Phosdrin, the interference of parathion can be greatly reduced and that of para-oxon is partly reduced by eluting a chloroform extract through activated carbon columns, because Phosdrin comes through the column earlier than either parathion or para-oxon (Sanjean *et al.* 1960).

Another example shows that aldrin can be bioassayed in the presence of dieldrin. After treating house flies with aldrin, dieldrin is rapidly formed by the biological oxidation of aldrin. When such extract (in hexane) is passed through a Florisil column (or other suitable adsorbants), aldrin will be eluted earlier than dieldrin. By this technique 11 μg. of aldrin and 12 μg. of dieldrin were found in 10 females, as compared with 12μg. of aldrin and 10 μg. of dieldrin obtained by colorimetric analyses (O'Donnell *et al.* 1954, 1955). In both analyses, bioassay and chemical analyses agree fairly well.

Stability.—Since insecticides vary greatly in their stability under various conditions, the decomposition of one type of compounds can be used to reduce the interference on the bioassay of another type of compounds. The following examples illustrate two kinds of instability which could be used, in combination with chromatography and insect toxicity, for the specific bioassay of aldrin, dieldrin, and endrin.

It was reported by Sun (1957) that by refluxing with a 6.5% alcoholic potassium hydroxide solution at 75° to 80° C. for 2 hours, aldrin and dieldrin (also endrin) are stable. By the same treatment toxaphene and chlordane are less stable and DDT, lindane and parathion give no interference in bioassay. Because of higher toxicity to house flies or vinegar flies of aldrin, dieldrin and endrin, chlordane and toxaphene would be expected to give relatively low interference after alkali treatment.

In field experiments, more than one insecticide is often used for practical control of various pests on one crop. The above technique of alkaline treatment has been applied to the recovery of aldrin and dieldrin in check samples contaminated with DDT, TEPP or parathion. Bioassay results indicate that 80% to 100% recoveries of fortified aldrin or dieldrin have been obtained in apple, celery, peaches and/or cherries at 0.1 or 0.3 p.p.m. level.

Another type of instability is that the toxicity of endrin can be destroyed on adsorptive type of dust diluents having high acidity on the clay surface (Fowkes *et al.* 1960). This property can be used when the residue of aldrin or dieldrin is bioassayed in the presence of endrin.

RESULTS OF SPECIFIC BIOASSAY.—To test the specific bioassay of Phosdrin in the presence of other organophosphorus insecticides, three characteristic properties have been found to reduce their interferences: (1) high toxicity of Phosdrin to *Drosophila*, (2) high speed of action, and (3) selective masking effect of plant extractives. This has been illustrated by the following experiments:

To the celery extract (without cleanup) add 1 p.p.m. of Phosdrin alone or in combination with 3 p.p.m. of one to four of the following organophosphorus insecticides—parathion, para-oxon, malathion, Guthion, Trithion, demeton, TEPP, and ethion. Using the same bioassay procedure, as described in a previous section, the toxicity results are recorded at 4 hours after exposure and compared with that of the Phosdrin standard curve prepared from an untreated extract. The average recovery of three determinations varies from 0.99 to 1.2 p.p.m. (table 3). The interference varies from 6.7% for parathion or para-oxon to 1.7% for a Trithion-demeton-TEPP-ethion mixture.

To test the degree of specificity of three analytical methods, celery extracts were first fortified with Phosdrin, parathion, para-oxon, Diazinon, Phosdrin plus parathion, Phosdrin plus Diazinon, and Phosdrin plus para-oxon. After carbon column and *n*-hexane-water partition cleanup described by Sanjean *et al.* (1960), each mixture

Table 3.—Bioassay of fortified Phosdrin in celery extracts in the presence of one, two or four other organophosphorus insecticides (without cleanup).

SAMPLE[a]	APPARENT PHOSDRIN FOUND (P.P.M.)			
	1	2	3	Mean ± S.E.
Phosdrin, 1 plus	0.96	1.02	1.05	1.0 ±0.026
Parathion, 3	1.39	1.09	1.21	1.2 ±0.087
Para-oxon, 3	1.15	1.17	1.32	1.2 ±0.056
Parathion, 3 and para-oxon, 3	1.34	1.18	1.13	1.2 ±0.064
Malathion, 3 and Guthion, 3	0.97	1.03	0.96	0.99 ±0.022
Trithion, 3, demeton, 3, TEPP, 3 and ethion, 3	1.14	1.28	1.17	1.2 ±0.045

[a] Toxicant given in p.p.m.

Table 4.—Comparison of three analytical methods on the determination of Phosdrin, parathion, para-oxon, Diazinon and their mixtures in celery extracts after carbon column and *n*-hexane-water partition cleanup.

RECOVERY SAMPLES		"PHOSDRIN" FOUND (P.P.M.)		
Toxicant Added	p.p.m.	Diazo Method	Cholinesterase Method	Bioassy Method
Phosdrin	0.15	0.20	0.22	0.16
	0.30	0.10	0.23	0.27
Parathion	1.5	−0.075	−0.015	<0.03
	15	0.0	0.0	0.06
Para-oxon	3	0.15	3.3	0.16
	20	0.0	13.8	0.66
Diazinon	3	0.03	0.0	0.03
Phosdrin+parathion	0.15+1.5	0.17	0.22	0.15
Phosdrin+para-oxon	0.3+ 3	0.14	0.23	0.48
Phosdrin+Diazinon	0.3 +3	0.0	0.33	0.29

Table 5.—Proposed schemes for specific bioassay of Phosdrin, aldrin, dieldrin and endrin residues.[a]

Toxicant	Extract and Treatment	Test Insect	Approximate Sensitivity (p.p.m.)	Insecticides Giving Low Interference	Crops Tested (or applicable)
Phosdrin	Chloroform: (a) No cleanup	Vinegar fly	1	Parathion, para-oxon, methyl para-oxon malathion, Diazinon, Trithion, Guthion, demeton, TEPP, ethion, Sevin	Celery, peppers, grapes, beets, boysenberries, carrots, squash
	(b) Activated carbon column	Vinegar fly	0.1	Parathion, para-oxon, methyl para-oxon, malathion, Diazinon, Trithion, Guthion, demeton, TEPP, ethion, Sevin	Orange, celery
Aldrin	*n*-Hexane or *n*-hexane-IPA; alkali treatment and column chromatography	Vinegar fly or house fly	0.1	BHC, lindane, DDT, chlordane, toxaphene, SD 4402[b], heptachlor, dieldrin, endrin, and organophosphorus insecticides	Apples, celery, cherries, peaches, corn, olives, wheat
Dieldrin	*n*-Hexane or *n*-hexane-IPA; treatment with alkali and Velvex, and column chromatography	Vinegar fly or house fly	0.1	BHC, lindane, DDT, chlordane, toxaphene, SD 4402, heptachlor, aldrin, endrin, and organophosphorus insecticides	Apples, celery, cherries, peaches, corn, olives, wheat
Endrin	*n*-Hexane or *n*-hexane-IPA; treatment with alkali and column chromatography	Aphids	—	BHC, lindane, DDT, chlordane, toxaphene, SD 4402, heptachlor, aldrin, dieldrin and organophosphorus insecticides	

[a] Dry-film method except topical (dip or spray) for endrin.
[b] 1,3,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan.

was then analyzed with cholinesterase (Hestrin 1949), diazo (Yipp 1959), and specific bioassay methods. Data in table 4 indicate that the cholinesterase method gives good recovery for Phosdrin and low interference caused by other organophosphorus insecticides, except para-oxon. Similar interference may also be expected with oxygen analogues of other phosphorothioates. Diazo method is not interfered by the organophosphorus compounds tested, but gives variable results for Phosdrin. Using the same extracts and the same cleanup methods, the specific bioassay method gives consistently satisfactory recoveries with low interference from other organophosphorus insecticides.

Conclusion and Discussion. – Many physical, chemical and biological means can be used for the separation and determination of a toxicant in a specific bioassay. Some of them have been discussed in this paper. Results indicate that Phosdrin residues can be determined by a bioassay method in the presence of any one or a mixture of two or four of the 11 cholinesterase-inhibiting insecticides with interferences of less than 7% of apparent Phosdrin (table 3). The specificity of this method depends largely upon the high toxicity and quick action of Phosdrin to vinegar flies, as well as the relatively low masking effect of plant extractives on the toxicity of Phosdrin. A proper cleanup with activated carbon and/or *n*-hexane-water partition can increase the sensitivity and further reduce the interference of some materials.

In addition to Phosdrin, schemes are proposed for the specific bioassay of aldrin, dieldrin and endrin residues (table 5). Since they are alkali stable, the toxicity of other insecticides may be partially or completely destroyed by alkali treatment. After the alkali treatment, the mixture is diluted with water and extracted with hexane. The concentrated hexane extract is then eluted through a Florisil column and aldrin comes out first. The toxicity of early fractions indicates the amount of aldrin and that of latter fractions indicates the presence of dieldrin and/or endrin. The separation of dieldrin from endrin can be accomplished by depositing the extract on an active clay, such as Velvex. After the evaporation of solvent, endrin will be decomposed in several hours at room temperature (Fowkes *et al.* 1960). Then the unchanged dieldrin is extracted from Velvex and bioassayed with house flies or vinegar flies. If endrin is the toxicant to be determined in a mixture of dieldrin and endrin, the mixture is bioassayed with aphids, such as pea aphids (*Macrosiphum pisi* (Harris)) because endrin is about 10 times as toxic as dieldrin to pea aphids.

These are only a few examples to illustrate the possibility of bioassaying one insecticide residue in the presence of others. If one investigates other physical and chemical means of separation and other species of insects for their differential reactions to different insecticides, more specific bioassay methods could be found for other insecticides.

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Resistance in Common Bean Lines to the Potato Leafhopper[1]

DAN WOLFENBARGER and J. P. SLEESMAN,[2] *Ohio Agricultural Experiment Station, Wooster*

ABSTRACT

Two common bean lines were resistant to nymphal infestation while 28 were intermediate in resistance to mean nymphal attacks. Ten lines exhibited no hopperburn injury while varying in resistance to nymphal attacks indicating a tolerance in beans. Although a comparison of hopperburn ratings and nymphal infestations identify tolerant varieties, nymphal counts are the better method for evaluation of resistance. The hopperburn ratings and mean 1959–60 nymphal counts showed highly significant positive correlation coefficients. Regression coefficients indicated that 0.99 to 2.85 nymphs were associated with each hopperburn class increase of one.

The common bean (*Phaseolus vulgaris* L.) is subject to attack by the potato leafhopper (*Empoasca fabae* (Harr.)) resulting in hopperburn injury. DeLong (1938) described hopperburn of the common bean as a stunting of the plant and curling of the leaves into a tight rosette. The object of these investigations was to identify sources of resistance in the common bean[3] to nymphal attacks and hopperburn damage and to compare methods of evaluation.

Beyer (1922) observed that the Pea bean, Wells, and the Red Kidney showed a marked degree of resistance to the potato leafhopper. Dudley (1926) reported that the string, pole, and navy beans were severely injured and, if not killed outright, the yields were reduced. McFarlane & Rieman (1943) stated that London Horticultural, Idaho, Wisconsin, and Stringless Green Refugee exhibited little damage from leafhopper feeding whereas Tennessee Green Pod was very susceptible. Tissot (1932) found fewer leafhopper nymphs on Bountiful, Black Valentine, Refugee 1000-1, and Giant Stringless Green Pod than any of the other varieties evaluated. Gui (1945) in Ohio stated that snap beans of the Refugee type were less heavily populated by the leafhopper than were the Green Pod snap bean and the common kidney bean varieties. Gates (1945) in Nebraska stated that U. S. Refugee #5 and Idaho Refugee were practically free of potato leafhopper nymphs while significantly greater numbers of nymphs were present on Landreth and Asgrow Stringless Green Pod. In Ohio, Guevara (1957) reported that Fullgreen ×Tendergreen, Contender, B2920-2, B3033-7-2, Seminole, Top Crop, Pearlgreen, B2446-1-6-1-3, and B2194-1-1 were resistant to hopperburn according to a relative, visual-rating scale. He also stated that U. S. Refugee #5, Seminole, and Idaho Refugee were low in resistance, whereas Stringless Black Valentine, Tenderlong-15, Burpee's and Landreth's Stringless Green Pod, Kinghorn Special, B1788-21-1, B3119-3, White Seeded Tendergreen, and Full Measure were susceptible either to nymphal infestation or to hopperburn. Hoffman (1959) and Tidd (1959) stated that Tenderlong-15 was susceptible to hopperburn.

MATERIALS AND METHODS.—All varieties and accessions were planted at Marietta and Wooster in single 5-foot rows with 3 feet separating the plots within and between the rows. Twenty to forty seeds were planted in each plot.

The methods used in estimating leafhopper infestation were (1) to count nymphs on trifoliate leaf samples selected from each plot and (2) to rate plots for leafhopper damage. Wolfenbarger (1961) described a relative hopperburn damage scale whereby lines which are rated 0 are considered highly resistant, and lines which are rated 4 are considered highly susceptible with intermediate values intermediate in resistance or susceptibility.

RESULTS AND DISCUSSION.—In tables 1 and 2 the nymphal infestations and the corresponding classes of hopperburn damage in lines of *P. vulgaris* are ranked from low to high. Tables 1 and 2 also show the variation in nymphal infestation and hopperburn damage at the two locations for the 3 years (1958–60) these lines were evaluated.

In this paper, resistance in beans to the potato leaf-

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[2] Research Assistant and Professor, respectively.

[3] Seed used in these investigations were supplied by A. B. Strand, Dandridge, Tennessee; J. C. Hoffman, Southeastern Vegetable Breeding Laboratory, Charleston, South Carolina; Leland W. Hudson, Regional Plant Introduction Station, Pullman, Washington; D. D. Dolan, Plant Introduction Station, Agricultural Experiment Station, Geneva, N. Y.; Asgrow Seed Co.; Rogers Bros. Seed Co.; Northrup, King & Co.; and Ferry-Morse Seed Co.

Table 1.—Reaction of *P. vulgaris* varieties or accessions to the potato leafhopper, Marietta and Wooster, Ohio. 1958–60.

Accession, Strain, or Variety	Nymphs per Trifoliate Leaf						Class Hopperburn[c]			Mean Nymphs	Mean Class Hopperburn[c]
	Marietta			Wooster			Wooster				
	1958	1959	1960	1958	1959	1960	1959	1960	Marietta 1959		
	July 16–19[a]	July 6–8[b]	July 11–12[a]	Aug. 7–12[a]	July 27–29[a]	Aug. 1–2[a]	Aug. 3	Aug. 16	July 23		
PI 151014	0.1	0	1.3	0.3	0.3	0	1.0	0		0.3	0.5
PI 173024	0.5	0.4	1.7	0	0	0	0	1.0	2.0	0.4	1.0
Puregold Wax	0.4	0.9		0.8					1.0	0.7	1.0
PI 174901	0.3	1.0	0.7	0.7	1.7	0.3	1.0	0	2.0	0.8	1.0
PI 169718	0.6	0.3	1.3	0.3	2.7	0	1.0	0	1.0	0.8	0.8
PI 136741	0.7	1.0	0.7	0.7	1.3	1.0	1.0	0		0.9	0.5
Stringless Blue Lake 231			0			1.7		2.0		0.9	2.0
U. S. #5 Refugee	0.3	1.2	2.7		0.7	0.3	1.0	0	1.0	1.0	0.5
Selected Canadian Wonder			1.7			0.3		1.0		1.0	1.0
Masterpiece			2.0			0		0		1.0	0
PI 172035	0.4	2.3		0.3					3.0	1.0	3.0
Corneli 14	0.2	1.0	0.7	1.4	1.0	2.0	1.0	0		1.1	1.5
Florida Belle	0.6	5.0	0	0	0.7	0.3	1.0	0		1.1	0.5
PI 169733	0.5	1.3	1.0	0.7	0	3.3	1.0	1.0	3.0	1.1	1.7
Sweetheart	0.1	0.7	0	1.2	4.7	0.3	1.0	0		1.2	0.5
Spartan			0			2.3		1.0		1.2	1.0
PI 169731	1.6	0.3	3.0	1.0	0.3		0		2.0	1.2	1.0
PI 171799	1.3	4.4	0.7	0.7	0.3	0	0	1.0	3.0	1.2	1.3
Seminole	0.4	5.9	0.3	0.3	0.7	0.3	0	0		1.3	0
Idaho Refugee	0.1	0.9	6.3	0	0.3	0	0.5		0	1.3	0.3
Stringless Red Valentine	0.5	1.9	2.0	0.6	1.3	1.3	2.0	0		1.3	1.0
PI 226938	0.6	2.1		1.3			1.0			1.3	1.0
Top Crop	1.5	0.9	1.7	1.4	2.3	0.7	2.0	0		1.4	1.0
Improved Supergreen	1.2	1.3	1.3	0.7	3.3	1.0	1.0	0	1.0	1.5	0.8
PI 179402	0.9	0.4	4.7	0.7	1.7	0.7	1.0	0	3.0	1.5	1.3
PI 207205	0.9	4.0	0	0.3	3.3	0.3	1.5	0	3.0	1.5	1.5
Top Notch Golden Wax	0.6	3.7		0.8	3.0	0	1.0	1.0		1.6	1.0
PI 172019	0.3	7.0	0.7	0.3	1.0	0	1.0	0	3.0	1.6	2.0
Improved Topcrop	0.5	0.4	2.3	1.4	3.7	1.3	1.0	0	1.0	1.6	0.8
Cherokee Wax	1.2	0.7	3.4	1.0	1.7	2.0	2.0	0.5		1.7	1.3
NK 105	2.9	0	1.0	1.4	3.3	1.3	1.0	0		1.7	0.5
Topmost	0.7	0		1.4	6.3	0	1.0	0.5	1.0	1.7	0.8
White Seeded Tendergreen	1.7	0.7	3.9	2.0	2.7	0	1.5	1.0	2.0	1.8	1.5
Contender	1.8	1.7	2.0	0.8	3.7	1.0	2.0	0		1.8	1.0
Green Crop (N.H. 23)	1.5	2.6	2.0	1.2	3.3	0	1.0	0		1.8	0.5
Choctaw	1.0	4.3	3.7	0.2	1.3	0.3	1.0	0		1.8	0.5
Cooper Wax	1.0	3.0	3.3	1.2		0.3		0	0	1.8	0
PI 172031	0	1.6	5.7	0	2.0	2.0	1.0	2.0	3.0	1.9	2.0
PI 172036	1.6	1.0	6.3	1.7	0.3	0.3	0	2.0	3.0	1.9	1.7
PI 209807	0.7	1.0	5.7	0.3	3.0	1.3	1.5	1.0	3.0	2.0	1.8
Tender and True			2.0			2.0		2.0		2.0	2.0
Comtesse de Chambord			2.3			1.7		0		2.0	0
Blue Ribbon			3.0			1.0		0		2.0	0
Improved Commodore	2.1	4.0	0	0.8	2.0	3.7	1.0	1.0		2.1	1.0
PI 169732	1.1	2.0	0	4.0	4.7	0.7	1.5	0		2.1	0.8
PI 183069	0.9	6.0	0.3	1.7	2.3	1.3	1.5	1.0	1.0	2.1	1.2
PI 180730	1.9	2.0	5.3	1.0		1.0		3.0	4.0	2.2	3.5
PI 182026	1.0	1.4	2.0	1.4	1.7	6.0	1.0	3.0	3.0	2.3	2.3
PI 209805	2.1	0.9	8.7	0.7	1.3	0	1.0	0	1.0	2.3	0.8
Glades	0.5	8.3	1.3	1.0	1.7	1.4	1.5	1.0	1.0	2.4	1.2
Pearlgreen	0.6	0.9	5.7	1.0	5.3	0.7	2.0	0	2.0	2.4	1.3
Tenderwhite	1.6	4.6		0.8	2.7		2.0		2.0	2.4	2.0
Fullgreen×Tendergreen	1.8	1.6	4.0	2.2			0			2.4	0
PI 171795	1.1	10.9	0.7	0.3	1.3	0	1.5	0	1.0	2.4	1.2
PI 182000	2.6	1.9	7.0	0	0.3	2.7	0.5	3.0	4.0	2.4	2.5
Sulfur			4.0			1.0		1.0		2.5	1.0
Kentucky Wonder Wax			4.3			0.7		1.0		2.5	1.0
Earligreen	2.1	2.9	5.0	2.0	2.3	1.3	0.5	0	1.0	2.6	0.5
Pea Bean			10.0			4.0		3.0		7.0	3.0
PI 150408	7.1	9.1	9.0	4.8	7.7	4.7	3.0	4.0		7.0	3.5
PI 150413	7.0	9.6	6.0	2.0	13.0	4.0	3.0	3.0		7.9	3.0
Stringless Blue Lake 228			11.7			4.3		2.0		8.0	2.0

(Continued on next page)

[a] 3 trifoliate leaves per plot.
[b] 7 trifoliate leaves per plot.
[c] Based on 0 least, 4 most.

Table 1.—(*Continued*)

Accession, Strain, or Variety	Nymphs per Trifoliate Leaf						Class Hopperburn[c]			Mean Nymphs	Mean Class Hopperburn[c]
	Marietta			Wooster			Wooster				
	1958	1959	1960	1958	1959	1960	1959	1960	Marietta 1959		
	July 16–19[a]	July 6–8[b]	July 11–12[a]	Aug. 7–12[a]	July 27–29[a]	Aug. 1–2[a]	Aug. 3	Aug. 16	July 23		
PI 173046	0.7	11.4		1.0	19.7		3.5		3.0	8.2	3.3
Italian or Romano			12.7			3.7		1.0		8.2	1.0
Stringless Blue Lake FM-1			10.3			7.3		1.0		8.8	1.0
Morse's Pole 191 (U.S. #4)			15.0			2.7		3.0		8.9	3.0
PI 207389	7.3	20.7	9.7	6.4	6.7	3.0	2.5	4.0		9.0	3.3
Tennessee Green Pod	7.2	18.7	10.3	4.2	11.3	2.7	2.5			9.1	2.5
PI 209467	4.6	17.6		1.7	14.3		3.5		4.0	9.6	3.8
McCaslan Pole			18.7			1.7		1.0		10.2	1.0
Blue Cocco Bean			9.3			11.3		2.0		10.3	2.0
PI 206975	6.5	18.4		4.7	13.3		3.5		4.0	10.7	3.8
Oregon Giant			20.3			2.3		2.0		11.3	2.0
PI 206971	15.4	16.3		6.3	13.3		3.5		4.0	12.8	3.8
Colossal			17.3			11.3		4.0		14.3	4.0
FM-52			27.3			3.7		0		15.0	0
Earliest of All			30.0			3.3		1.0		16.7	1.0
Dutch Brown			20.0			19.3		2.0		19.7	2.0

Table 2.—Advanced breeding lines of *P. vulgaris* evaluated for resistance to the potato leafhopper, Marietta and Wooster, Ohio. 1958–60.

Variety	Nymphs per Trifoliate Leaf						Class Hopperburn[a]			Mean Nymphs	Mean Class Hopperburn[a]
	Marietta			Wooster			Wooster				
	1958	1959	1960	1958	1959	1960	1959	1960	Marietta 1959		
	July 16–19	July 6–8	July 11–12	Aug. 7–12	July 27–29	Aug. 1–2	Aug. 3	Aug. 16	July 23		
B3379	1.0	0.1	1.9	2.2	2.0	0.7	1.0	0		1.2	0.5
B3239-20-X	1.3	1.9	0.7	0.2	0.7	3.7	1.0	0	1.0	1.4	0.7
B3164-1-1-1	1.5	2.7		1.4	0.3		0.5		1.0	1.5	0.8
B3076	0.8	2.7	0	0.2	2.3	0.3	0	0		1.5	0
B3260-1	3.1	2.3	1.7	1.0	1.0	0.3	0	1.0		1.6	0.5
B2920-2			2.7		1.0	1.0	1.0	0		1.6	0.5
B3183-1-1	2.6	1.7	3.3	0.8	1.0	0	0	1.0	1.0	1.6	0.7
B2932	1.2	0.3	1.7	1.0	4.3	1.3	1.5	1.0	0	1.6	0.8
B3126-1	1.9	1.4	5.0	1.0	1.3	0.3	1.0	0	1.0	1.8	0.7
B2968-1	0	3.7	3.0	1.0	0.9	3.0	1.5	1.0	1.0	1.9	1.2
B3097-1-1-EC	1.2	2.0	3.0	2.2	2.3	1.0	1.0	1.0	1.0	2.0	1.0
B3441	0.9	4.3	2.0	1.2	2.7	1.7	1.5	0	1.0	2.1	0.5
B3121	1.6	2.4	2.0	1.8	3.0	1.7	1.0	1.0		2.1	1.0
B3504	1.3	1.7	3.3	0.6	5.3	0.7	1.0	0		2.2	0.5
B3340-1	3.1	0.9	1.0	2.8	1.7	3.7	1.0	0	1.0	2.2	0.7
B3093-1	2.1	6.0	0.3	1.4	2.3	1.3	1.0	1.0	1.0	2.2	1.0
B3505	1.7	2.7	2.0	1.8	3.0	1.0	1.0	0	1.0	2.3	0.7
B3365-2	2.4	0.6	4.0	1.0	4.3	1.7	1.0	1.0		2.3	1.0
B2948-2-1-2	2.4	1.8	1.7	3.7	3.7	0.5	1.0	1.0	1.0	2.3	1.0
B3094-1-3-1	0.8	4.3	0.3	2.0	3.3	4.0	2.0			2.5	2.0
B2761-X	1.9	3.3	4.0	3.3		0.7		0	2.0	2.6	1.0
B2523-2-1-2	3.8	1.3	4.7	2.8		0.3		0	1.0	2.6	0.5
B2720-4-1-EC	1.7		4.0	1.6		3.7		0		2.8	0
B3339-2	3.4	2.4	3.0	1.8	2.3	3.7	2.0	0		2.8	1.0
B2934-1-1-1	4.6	1.6	3.3	2.6		2.3		0	1.0	2.9	0.5
B3125-X-5-2	2.7	4.3	1.7	3.8	3.0	2.0	1.0	0		2.9	0.5
B3348	2.8		2.7	2.6		4.0		1.0		3.0	1.0
B3281-2-1	2.5	5.7	7.0	0.4	1.3	2.0	0	0		3.1	0
B2971-1-1	1.1	2.8	5.7	0.6	8.3	0	1.0	0	0	3.1	0.3
B2637-1-1-X	3.7	8.0	1.0	1.4	30.7	2.0	2.5	0		7.8	1.3
B3054-2-1-1		4.7	4.3		16.3	9.0	3.5	2.0	1.0	8.6	2.2
B3076-3		9.3	9.3		21.7	1.0	4.0	1.0	1.0	9.3	2.0
B3070-CB-1-X		8.7	10.0		25.3	1.3	4.0	1.0	2.0	11.3	2.3

[a] Based on 0 least, 4 most.

Table 3.—Mean leafhopper nymphal populations vs. the class of hopperburn ratings of *P. vulgaris* varieties and accessions, range of nymphs, Marietta and Wooster, Ohio. 1959–60.

Visual Rating Class	Mean Nymphs per Trifoliate Leaf			Mean Range Nymphs per Trifoliate Leaf		
	1959		1960	1959		1960
	Marietta	Wooster	Wooster	Marietta	Wooster	Wooster
0	4.6	1.3	1.7	0.5–25.9	0.2– 7.0	0.3–11.7
1	5.1	2.3	2.3	0.6–28.1	0.4–18.7	0.4–11.0
2	5.8	4.8	3.1	0.6–32.7	0.4–21.0	0.4–19.3
3	6.5	7.8	3.8	0.5–24.9	2.5–25.3	0.3– 9.0
4	10.1	14.7	5.9	0.7–31.4	1.7–25.3	0.7–22.6
No. of varieties evaluated	1,140	1,306	1,024			

hopper has been considered high if the mean number of nymphs per trifoliate leaf is 0.4 or less and low if the number is 0.5 to 1.5. All other mean nymphal infestations were considered susceptible. Only two *P. vulgaris* lines, PI 151014 and PI 173024, were resistant to nymphal infestations and both of them exhibited some hopperburn. Of the 1619 lines of *P. vulgaris* evaluated, 28 or 1.7% were low in resistance.

Freytag (1955) stated the pubescence of *P. vulgaris* consisted of hooked, simple, stellate, and glandular types of hairs. Wolfenbarger (1961) obtained a correlation coefficient of $r = -0.194$ (not significant at 5% probability level) between the number of epidermal hairs and the nymphal population of this variety. He observed that the number of epidermal hairs ranged from 96 to 492 per 0.4 mm. on 59 commercial varieties of *P. vulgaris*. These data indicate that the density of the hairs on the leaf surface may not be a factor in protecting the beans from attacks of the leafhopper. Perhaps the type of hair and not the number per unit is the important factor. Poos & Smith (1931) stated that young nymphs of *E. fabae* were frequently observed impaled on hooked hair types of Stringless Green Pod bean.

In table 3 the relative hopperburn damage scale is compared with the nymphal population. Correlation coefficients ($r = +0.90$, $+0.91$, $+0.96$ significant at the 1% level) and regression coefficients ($b = 1.26$, 2.85, 0.99) for 1959 at Marietta and Wooster and 1960 at Wooster, respectively, were computed. A linear correlation coefficient, $r = +0.55$ significant at the 1% level, was computed from the lines in table 2 and from 29 other advanced breeding lines (Wolfenbarger 1961). These data indicate a close relationship between nymphal population and the visual rating score. The three regression coefficients indicate that for every 0.99 to 2.85 nymphs per trifoliate leaf the hopperburn rating is increased by one. The F value of the regression coefficients was highly significant (0.01% level) indicating that the data was not homogeneous. The regression of nymphs on hopperburn ratings is real but varies from year to year and location to location.

Even though hopperburn ratings indicate the reaction of the plant to nymphal feeding and are closely correlated with nymphal populations, it is thought that a count of nymphs is the better method of evaluation because it shows whether the plant actually affects nymphal development or whether the constantly moving adults exhibit a preference in oviposition. However, a comparison of the two methods will determine whether or not the lines merely tolerate nymphal infestation.

According to tables 1 and 2 various lines, such as McCaslan Pole, FM-52, Stringless Blue Lake FM-1, Earliest of All, and B2637-1-1-X, had hopperburn ratings of 0 to 1.3 whereas the mean nymphal infestation ranged from 7.8 to 16.7. This would indicate a resistance to hopperburn in view of the fact that only 6.5 nymphs per trifoliate leaf would be enough to cause a Class 4 hopperburn rating.

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Interactions of the European Corn Borer and Stalk Rot in Corn[1]

H. C. Chiang and Roy D. Wilcoxson[2]

Abstract

Egg masses of corn borer (*Pyrausta nubilalis* (Hbn.)) and/or inoculum of *Fusarium graminearum* Schw. were introduced into artificial tunnels in corn stalks of single crosses WF9×M14 and Oh43×Oh51A growing in the field. The amount of necrotic tissue in stalks infested with corn borer larvae was always greater than when stalks were not infested. The presence of *F. graminearum* in tissue infested with corn borer larvae resulted in more rapid development of the larvae. Artificial inoculation of tunnels with *F. graminearum* had no apparent effect on numbers of corn borer larvae in the tunnels or the ability of the larvae to colonize the tunnels.

The European corn borer (*Pyrausta nubilalis* (Hbn.)) and stalk rot are major threats to production of corn (*Zea mays* L.) in the United States. In 1950 it was reported in Minnesota that severity and prevalence of stalk rot were greatly increased by activities and presence of the corn borer (Christensen & Schneider 1950). More work is needed on the nature of relationships between the insect and the stalk rot pathogens. With the discovery (Chiang 1959) that borers may be established in artificial tunnels in corn stalks, it became possible to manipulate both the insect and the plant pathogens so their mutual relationships could be studied closely. The present paper deals primarily with the effect of the corn borer on stalk rot and of *Fusarium graminearum* Schw. on the corn borer.

Materials and Methods.—The experiments were made on two single crosses of field corn: WF9×M14, susceptible to both corn borer and stalk rot, and Oh43×Oh51A resistant to corn borer and moderately resistant to stalk rot. The corn was grown at the Experiment Station at Waseca, Minnesota, in 1959 and 1960. The treatments were applied to plants about 1 week prior to tasseling.

Artificial tunnels were made in corn stalks using essentially the same method as described by Chiang (Chiang 1959). Leaf sheaths were removed and tunnels made in the centers of the two basal internodes with a 6.35-mm. bit and electric drill. The tunnels were angled slightly upward into the internodes to allow for drainage. In 1959 the tunnels were covered with putty but in 1960 they were plugged with cotton. In those experiments where it was desirable to produce tunnels aseptically, the corn stalk was washed with 70% ethyl alcohol and then drilled with a flamed bit. These tunnels were either plugged with putty or covered with a sterile band-aid, manufactured by Johnson and Johnson of New Brunswick, New Jersey.

Egg masses of corn borer averaging about 20 eggs per mass and carried on wax paper discs 12.5 mm. in diameter were used in this work. The egg masses were all in the blackhead stage, *i.e.*, the larvae were well formed and would be hatching within a few hours, at the time of introduction into plants. Hatching exceeded 90%. One egg mass was placed in each tunnel.

Cultures of *F. graminearum* were grown on autoclaved wheat kernels. When plants were inoculated, one kernel of wheat which was covered with mycelium was placed in the tunnel. Micro-organisms present in necrotic tissues were identified using standard laboratory methods.

At 2-, 3-, or 4-week intervals after tunnels were made notes were taken by cutting the plants longitudinally and estimating the percentage of necrotic tissue present in the internodes being studied. The tissues were then completely dissected and the developmental stage of borers present was determined. During the intervals stated, rarely did the insect penetrate or the necrosis extend into adjoining internodes.

In 1959 the following treatments were made: a) Aseptically produced tunnel, b) aseptically produced tunnel plus egg mass, c) septically produced tunnel, and d) septically produced tunnel plus egg mass. In 1960 aseptic techniques were not used for making the tunnels. The treatments were: a) tunnel alone, b) tunnel plus *F. graminearum*, c) tunnel plus egg mass, and d) tunnel plus *F. graminearum* and egg mass.

Results.—*Stalk Rot Studies.*—In a preliminary study made in the greenhouse in the spring of 1959 stalk tissues were kept free of necrosis for 10 days when tunnels were made in inbred lines of corn W22, A116, V3, and Oh51A by using an aseptic technique and covering the tunnel with band-aids. In tunnels made without aseptic techniques necrotic tissue was always present and was sometimes extensive. When similar procedures were followed in the field in 1959, however, all tissues around the tunnels were necrotic but the necrosis was less severe in tunnels free of insects.

The putty used for plugging tunnels in 1959 prevented drainage of fluids from the tissues and insect establishment was thereby greatly reduced. The larvae developed in enough of the stalks, however, to indicate that they markedly increase the amount of necrotic tissue in the corn stalks (table 1 and figure 1). The influence of the insects on formation of necrotic tissue was particularly striking 4 weeks after treatment. There was little variation in the amount of necrosis present in plants of a particular treatment. The following micro-organisms were isolated from the necrotic tissues: *F. graminearum*, *F. moniliformae* Sheld., *Diplodia zeae* (Schw.) Lev., and species of *Penicillium*, *Aspergillus*, *Alternaria*, *Rhizopus*, yeasts and bacteria.

In 1960, plants were inoculated with *F. graminearum* and infested with corn borer eggs. The presence of the insect in the tunnels greatly increased the severity of necrosis in corn tissue. The effect of the insect was apparent with each dissection of the plants but was most clearly seen on the last date (table 2). Data for the third

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[2] Professor, Department of Biology, University of Minnesota, Duluth, and Department of Entomology and Economic Zoology; and Assistant Professor, Department of Plant Pathology and Botany, University of Minnesota, St. Paul, respectively.

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Table 1.—Percentage of tissue that was necrotic in internodes of two single crosses of corn 2 and 4 weeks after infestation with corn borer, 1959.

CORN BORER PRESENCE	WF9×M14		Oh43×Oh51A	
	No. of Internodes[a]	Per Cent of Tissue Necrotic	No. of Internodes	Per Cent of Tissue Necrotic
2-week dissection				
Present	18	15	27	15
Absent	230	5	230	6
4-week dissection				
Present	13	42	31	49
Absent	230	9	230	11

[a] A total of 230 internodes were included in each treatment but the insect became established only in the number of internodes indicated.

week dissection are not presented because they were intermediate between the two sets of data shown. Inoculation tended to mask the influence of the insect but the tendency for inoculated tissues to be more necrotic in the presence of corn borer was apparent.

The single crosses were selected for this work because they have differed in stalk rot resistance in other tests, but no difference in resistance was apparent in these tests.

Insect Studies.—After the tissues had been evaluated for the amount of necrosis present, they were completely dissected and the number of corn borer larvae present and their stages of maturity were noted. Data concerning development of the larvae in 1960 are presented in figure 2, which shows the percentage of the larvae in various stages of development 2 weeks after the experiment began. Data for the other dates of observation were similar to these and are therefore not shown. Data presented in figure 2, show that mature larvae were more numerous in stalks of WF9×M14 than in Oh43×Oh51A, susceptible and resistant single crosses, respectively, in leaf reaction trials. This difference was not large, however.

The effect of having the larvae develop in the presence of *F. graminearum* is also indicated in figure 2. At the time when notes were taken and on each single cross of corn, a greater percentage of the larvae were more mature when feeding on tissues inoculated with the fungus.

On the average, slightly more than one larva was found associated with each tunnel on each date of observation. There were no apparent differences in the populations

Table 2.—Percentage of tissue that was necrotic in internodes of two single crosses of corn 2 and 4 weeks after inoculation with *Fusarium graminearum* and infestation with corn borer, 1960.

INOCULATED WITH *F. graminearum*	CORN BORER PRESENCE	WF9×M14		Oh43×Oh51A	
		No. of Internodes	Per Cent of Tissue Necrotic	No. of Internodes	Per Cent of Tissue Necrotic
2-week dissection					
Inoculated	+[a]	35	27	42	27
	−	60	24	52	25
Not inoculated	+	44	16	34	17
	−	52	10	58	11
4-week dissection					
Inoculated	+	24	29	25	27
	−	55	23	60	20
Not inoculated	+	32	25	24	23
	−	58	11	56	11

[a] + Indicates the corn borer was present in these internodes; − indicates internodes free of corn borer. A total of 60 internodes were in each treatment.

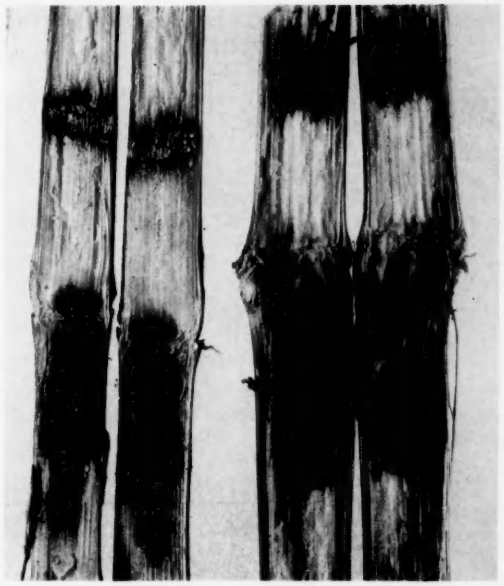

FIG. 1.—The increase in amount of necrosis in corn stalk tissues when corn borer larvae were present (lower pair of lesions) over necrosis where larvae were absent (upper pair of lesions).

that could be related to the single cross or to the presence of *F. graminearum*.

The success with which larvae colonized tunnels was high as indicated in table 3, where 44% to 73% of the tunnels were colonized. Introducing *F. graminearum* with the egg mass did not aid the larvae in establishing themselves, however.

The single crosses were chosen for this work because they differ in their resistance to corn borer in leaf reaction trials. This difference in resistance was not apparent from the ability of the larvae to establish themselves in artificial tunnels. However, the cross Oh43×Oh51A, considered to be resistant, did slightly retard the speed of development of the larvae when compared with WF9 ×M14, considered to be susceptible.

Table 3.—Number and percentage of artificial tunnels in stalks of two single crosses in which corn borer larvae were established 2 and 4 weeks after tunnels were made, 1960.

SINGLE CROSSES	INOCULATED WITH *F. graminearum*	NO. OF TUNNELS	TUNNELS WITH LARVAE	
			Number	Per Cent
2-week dissection				
Oh43×Oh51A	+[a]	58	42	72
	−	52	34	65
WF9×M14	+	60	35	58
	−	60	44	73
4-week dissection				
Oh43×Oh51A	+	51	25	49
	−	54	24	44
WF9×M14	+	47	24	51
	−	60	32	53

[a] + indicates the internodes were inoculated; − indicates the internodes were not inoculated. A total of 60 internodes were in each treatment.

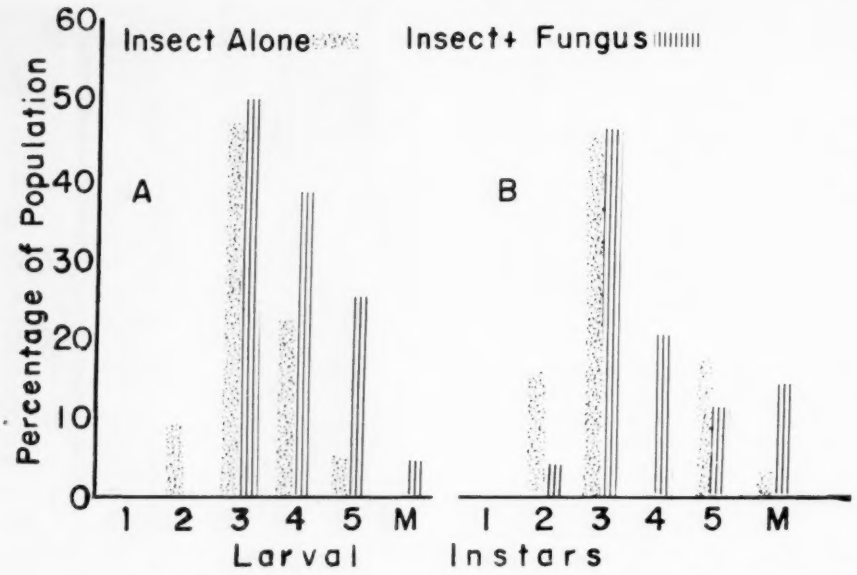

FIG. 2.—Percentage of population of corn borer larvae in various instars in stalks of corn single crosses Oh43×Oh51A (A) and WF9 ×M14 (B). The insect was allowed to develop alone or in the presence of *Fusarium graminearum*.

DISCUSSION.—Development of corn borer larvae was more rapid in tissue infected with *F. graminearum*. This suggests that micro-organisms may either aid the insect in utilizing corn stalk tissue or provide some essential nutrient. More work is needed on the nutritive requirements of the corn borer and the role of microorganisms in supplying nutrients.

It is not known whether leaf and stalk tissues of corn plants are equally resistant to the corn borer. In this work the single crosses were used because they differed in their corn borer resistance as judged by leaf feeding tests. However, when the larvae were placed in the stalk tissues, they were able to establish themselves about as well in one single cross as in another. In stalk tissues of a susceptible single cross, WF9×M14, development of the larvae was slightly more rapid than in stalks of the resistant single cross, Oh43×Oh51A. On the other hand, when plants of Oh43×Oh51A were inoculated with *F. graminearum* the larvae developed more rapidly than in the uninoculated susceptible single cross, WF9×M14.

The experiments reported in this paper clearly demonstrated that corn stalks developed more necrotic tissue when infested with corn borer larvae; the necrosis being due to various fungi, yeast, and bacteria. The relationship between the corn borer and stalk rot organisms should be taken into consideration in breeding for resistance to either corn borer or stalk rot.

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PUBLICATION POLICIES OF THE ENTOMOLOGICAL SOCIETY OF AMERICA AND MANUSCRIPT RULES AND SUGGESTIONS

A revision of this paper appeared in the Bulletin of the Entomological Society of America Volume 7, Number 2, pp. 59–62, June, 1961. Authors and coauthors of manuscripts intended for publication by the Society should consult this revision carefully before preparing such manuscripts for publication.

Evaluation of Insecticides for the Control of the Grape Berry Moth, *Paralobesia viteana*[1]

JAMES A. COX, *Pennsylvania Agricultural Experiment Station, University Park*

ABSTRACT

Field tests from 1957–1960 for control of the grape berry moth, *Paralobesia viteana* (Clemens) included a spray schedule of two sprays for the first brood and one for the second. The first application was made soon after the berries set, the second 10 days later and the final spray in August. Sprays of parathion, Guthion® (*O,O*-dimethyl-*S*-4-oxo-1,2,3-benzotriazin-3-(4-H)-ylmethyl phosphordithioate), Diazinon® (*O,O*-diethyl *O*-(2-isoprophyl-6-methyl-4-pyrimidyl) thiophosphate), and Sevin® (1-naphthyl *N*-methylcarbamate) were as effective as the standard DDT. DDT in the final spray in August is likely to leave excessive spray deposits on the fruit at harvest. Insecticides that may be substituted for DDT in the final spray are: parathion, methoxychlor, Diazinon, Sevin and Guthion. These insecticides applied in a split program with DDT are equally as effective as a straight schedule of DDT and the residue on the fruit at harvest is well within the legal tolerance.

Previous investigations by Cox (1949) and Taschenberg (1950) have shown that DDT is very effective against the grape berry moth, *Paralobesia viteana* (Clemens). For a number of years in the Erie Grape Belt of Pennsylvania, the spray program for the control of this insect has been three past-blossom sprays of DDT. The first application is made soon after the berries set, followed by a second 10 days later. The final spray is applied in August. Although this program has been highly effective against the grape berry moth, it has not been entirely satisfactory from the standpoint of spray residues. Previous investigations by Cox (1949) and Taschenberg & Avens (1949), have shown that DDT in the final spray in August is likely to leave excessive spray deposits on the fruit at harvest. In recent years control studies have been under way to obtain information on some of the newer insecticides and to find a substitute for DDT in the final spray. This paper reports the work on control studies of the grape berry moth from 1957 to 1960. The results of tests on residue studies of the several spray programs will be published elsewhere.

METHODS AND MATERIALS.—The investigations were conducted in commercial vineyards on the variety Concord. The dilute sprays were applied to plots that were five rows wide and 18 to 24 vines long. Where the concentrate sprays were tested the plots were seven rows wide and 18 to 30 vines long. In all the tests the treatments were replicated three times. The spray schedule followed was two sprays for the first brood and one for the second. The first application was made soon after blossoming, the second 10 days later, and the third in August.

The dilute sprays were applied with a hydraulic sprayer equipped with a covered, inverted, U-shaped boom carrying seven nozzles on each side of the row. The spray was applied at a pressure of 450 to 500 p.s.i. The concentrate spray was applied with an air-blast machine. The air was generated by a double 18-inch blower. The spray was injected into the air stream through a nozzle that produced a flat spray, at a pressure of 125 p.s.i. Two fish-tail air outlets directed the spray upwards and into the foliage and clusters of the vine.

The treatments were evaluated during harvest by selecting 10 vines from the center row of each plot. Ten clusters were taken from each vine to obtain a record of the infestation. These clusters were removed from the vine for examination. On the untreated vines a record was made of the number of injured and uninjured berries on the clusters. Only the injured berries were counted on the treated clusters. The average number of berries per cluster was determined from counts on the untreated. The results were converted to a percentage basis and the per cent control was calculated according to the formula of Abbott (1925).

The materials used in the experiments were standard brands of insecticides. In addition to those included in the list of common names of insecticides by Smith (1959), the following were included in the tests reported here: Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thioposphate), dimethoate, Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate), Sevin® (1-naphtyl *N*-methylcarbamate), Perthane® (1-1 dichloro-2-2 bis (*p*-ethylphenyl)ethane), Phaltan® (N-trichloro-methyl mercaptophthalimide), *Bacillus thuringiensis* (Berliner) (a microbial insecticide containing 75 billion spores per gram), Sovaspray Oil No. 3 (viscosity 58–64 seconds Sayboldt at 100° F., unsulfonated residue 70% to 83%, Socony-Vacuum Oil Co.), Triton B-1956 (pthalic anhydride of glycerol alkyl resin, Rohm and Haas Co.), miscible oil (containing 9 parts of Sovaspray Oil No. 3 and 1 part of Triton B-1956), tribasic copper sulphate (containing 26% metallic copper).

RESULTS AND DISCUSSION.—During 1957 and 1958, tests were performed in vineyards to compare the efficiency of certain insecticides and several schedules of DDT and other compounds, applied as concentrate sprays. In 1957 the first and second sprays were applied with ferbam at 6–100 rate and 1 pint of miscible oil. In the final spray all treatments except number 8 were applied with tribasic copper sulphate 12 pounds, lime 12 pounds and 1 quart of miscible oil. Treatment 8, in the third spray, was applied with ferbam at 6–100 rate and 1 pint of miscible oil. The results are given in table 1A. It is readily apparent that three applications of DDT, Diazinon, Guthion, Sevin, ethion, and a split program of two sprays of DDT followed by one application of methoxychlor, parathion, Perthane or Diazinon gave satisfactory control.

In 1958 the infestation was light and the results from only one vineyard are worthy of consideration. In this vineyard a comparison was made of DDT and Sevin against the insect. The first and second sprays contained ferbam at 6–100 rate and 4 ounces of commercial sticker. In the third application Sevin (treatment 12) was applied

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Table 1.—Efficiency of sprays for the control of the grape berry moth, 1957, 1958, 1959, and 1960.

Treatment No.	Material and Amounts per 100 Gallons	Applications	Per Cent Infested	Per Cent Control
	A. 1957[a]			
1	DDT 50%, W.P., 6 lb.	1, 2 & 3	0.6	97.4
2	DDT 50%, W.P., 6 lb. Methoxychlor, 50% W.P., 8 lb.	1 & 2 3	0.4	98.2
3	DDT 50%, W.P., 6 lb. Perthane 50%, W.P., 8 lb.	1 & 2 3	1.0	95.8
4	DDT 50%, W.P., 6 lb. Parathion 42%, E.C., 1 qt.	1 & 2 3	0.4	98.4
5	Diazinon 25%, W.P., 8 lb.	1, 2 & 3	0.6	97.5
6	Guthion 15%, W.P., 6 lb.	1, 2 & 3	1.0	95.9
7	Ethion 25%, W.P., 8 lb.	1, 2 & 3	0.7	96.8
8	Sevin 50%, W.P., 8 lb.	1, 2 & 3	1.1	95.4
9	DDT 50%, W.P., 3 lb. Diazinon 25%, W.P., 3 lb.	1, 2 & 3	0.7	97.0
10	DDT-parathion,[b] 6 lb.	1, 2 & 3	0.7	97.2
11	Untreated		21.5	
	B. 1958[c]			
12	Sevin 50%, W.P., 8 lb.	1, 2 & 3	1.8	89.0
13	Sevin 50%, W.P., 8 lb.	1, 2 & 3	0.3	98.2
14	DDT 50%, W.P., 6 lb.	1, 2 & 3	1.1	92.1
15	Untreated		16.4	
	C. 1959[d]			
1	DDT 50%, W.P., 1.5 lb.	1, 2 & 3	3.6	84.4
2	DDT 50%, W.P., 1.5 lb. Sevin 50%, W.P., 1 lb.	1 & 2 2	2.0	91.1
3	DDT 50%, W.P., 1.5 lb. Diazinon 25%, W.P., 2 lb.	1 & 2 3	1.1	95.4
4	Sevin 50%, W.P., 1 lb.	1, 2 & 3	1.4	93.8
5	Sevin 50%, W.P., 2 lb.	1, 2 & 3	1.1	95.4
6	Dimethoate 25%, W.P., 2 lb.	1, 2 & 3	1.2	94.8
7	Ethion 25%, W.P., 2 lb.	1, 2 & 3	1.2	94.8
8	Guthion 25%, W.P., 1 lb.	1, 2 & 3	0.9	96.3
9	DDT 50%, W.P., 1.5 lb. *Bacillus thuringiensis*, 1 lb.	1 & 2 3	12.2	46.9
10	DDT 50%, W.P., 6 lb. Sevin 50%, W.P., 4 lb.	1 & 2 3	1.0	95.7
11	DDT 50%, W.P., 6 lb.	1, 2 & 3	1.6	93.2
12	Untreated		22.9	
	D. 1960[e]			
	Vineyard A			
1	Sevin 50%, W.P., 2 lb.	1, 2 & 3	0.4	97.9
2	Sevin 50%, W.P., 1 lb.	1, 2 & 3	0.8	95.9
3	Dimethoate 25%, W. P., 1 lb.	1, 2 & 3	3.7	81.1
4	Dimethoate 25%, W.P., 2 lb.	1, 2 & 3	5.8	70.4
5	Ethion 25%, W.P., 2 lb.	1, 2 & 3	0.9	95.5
6	Guthion 25%, W.P., 1 lb.	1, 2 & 3	0.2	98.9
7	Untreated		19.6	
	Vineyard B[f]			
8	DDT 50%, W.P., 2 lb.	1 & 2		
	Sevin 50%, W.P., 2 lb.[g]	3	0.1	99.3
9	DDT 50%, W.P., 2 lb. Parathion 15%, W.P., 1.5 lb.	1 & 2 3	0.3	97.7
10	DDT 50%, W.P., 3 lb. Methoxychlor 50%, W.P., 2 lb.	1 & 2 3	0.2	98.7
11	DDT 50%, W.P., 2 lb. Diazinon 25%, W.P., 2 lb.	1 & 2 3	1.4	90.1
12	DDT 50%, W.P., 2 lb. Parathion 79%, E.C., 4 oz.	1 & 2 2	0.7	94.8
13	Untreated		14.2	

[a] Sprays applied June 20, July 2 and August 7 at the rate of 65 to 75 gal./A., 4×concentration.

[b] Black Leaf 253 W.P. containing 25% DDT and 3% parathion. Product of Virginia-Carolina Chemical Corporation.

[c] Sprays applied July 1, 14, and August 12 at the rate of 65 to 75 gal./A., 4×concentration

[d] Sprays applied June 18, 29, and August 4 at the rate of 250 to 300 gal./A.

[e] Sprays applied June 21, 30, and August 12 at the rate of 250 to 300 gal./A.

[f] Sprays applied June 22, July 2 and August 11 at the rate of 250 to 300 gal./A.

[g] Tribasic copper sulphate 3 pounds, lime 3 pounds and 1 quart of miscible oil applied July 26.

with tribasic copper sulphate 12 pounds, lime 12 pounds and 6 ounces of commercial sticker. Treatments 13 and 14 were applied with Phaltan at 8–100 rate and 4 ounces of commercial sticker. It is well known that Sevin is not compatible with lime. However, it seemed desirable to test this combination, since copper is desirable in the final spray for the control of certain diseases. As shown by the data presented in table 1B, Sevin applied with a mixture of lime and copper did not give a high degree of control. Sevin applied with ferbam and Phaltan gave satisfactory control and was as effective as DDT. Recently, Taschenberg, *et al.* (1960) have shown that the effectiveness of Sevin against the grape berry moth is greatly reduced by the presence of lime in the spray mixture.

The results obtained in 1959 with various spray schedules are shown in table 1C. In these tests, treatments 1 to 9, in the first spray contained ferbam at 1.5–100 rate and 2 ounces of commercial sticker. The second and third applications contained Phaltan at 2–100 rate and 4 ounces of commercial sticker. Treatments 10 and 11 were applied at 4x concentration and in the first spray contained ferbam at 6–100 rate and 2 ounces of commercial sticker. The second and third applications contained Phaltan 6–100 rate and 4 ounces of commercial sticker. In these experiments three applications of DDT did not give a high degree of control. Sprays of Guthion, ethion, dimethoate, Sevin and split schedules of DDT and Sevin and DDT and Diazinon were quite effective against the insect. A schedule of two applications of DDT against the first brood and one spray of *Bacillus thuringiensis* for the second did not control the insect.

A summary of the results obtained in 1960 is given in table 1D. In vineyard A, all treatments contained Phaltan 2–100 rate and 2 ounces of commercial sticker. In vineyard B, the first and second applications contained ferbam 1.5–100 rate and 2 ounces of commercial sticker. In the third spray all the treatments except No. 8 were applied with tribasic copper suphate 3 pounds, lime 3 pounds and 1 quart of miscible oil. It is readily apparent (vineyard A) that three applications of Sevin, Guthion or ethion gave satisfactory control. Sevin at 1–100 rate gave remarkably good control. Three applications of dimethoate were not entirely satisfactory. It is quite obvious that these results are not in agreement with the previous year's data. It should be pointed out that the dimethoate formu-

lation for 1960 was in question and this may have had some influence on the results. As indicated by the results given in table 1D (vineyard B), a split program of two sprays of DDT for the first brood and a final spray of parathion, Sevin or methoxychlor for the second gave excellent control of the insect.

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Predisposition to Pythium Seedling Disease and an Activated Charcoal-Fungicide Interaction as Factors Influencing Emergence of Cotton Seed Treated with Phorate[1]

D. C. ERWIN, H. T. REYNOLDS, and M. J. GARBER,[2]

University of California Citrus Experiment Station, Riverside

ABSTRACT

Seed treated with Ceresan 100 or 200 (mixtures of ethyl mercury 2,3-dihydroxypropyl mercaptide and ethyl mercury acetate) and subsequently treated with proprietary phorate (formulated as 44% chemical on charcoal) showed less protection against pre-emergence damping-off in unsterilized soil than seed treated only with Ceresan. This effect was more evident and severe at soil temperatures of 16° C. than at 21° to 24° C. No reduction in emergence of seed treated with phorate-Ceresan occurred in steamed pathogen-free soil except when it was artificially infested with pathogenic *Pythium* spp. In naturally infested soil, treatment of seed with charcoal in addition to Ceresan reduced emergence but not so severely as treatment with phorate-Ceresan. This suggested that the charcoal carrier interacted with Ceresan and that the active chemical in phorate predisposed the seed to pathogenic *Pythium* spp. The emergence-reducing effect was less marked at 2 pounds of proprietary phorate per 100 pounds of seed than at 8 pounds. When seeds treated with Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithicate), a similar systemic insecticide, and Ceresan were planted in naturally infested soil, the emergence was not reduced. Emergence was delayed, however, and seedlings were temporarily stunted in steamed pathogen-free soil.

The organophosphorus insecticide phorate (sold commercially as Thimet® 44D) has shown promising insecticidal action on cotton for 6 to 8 weeks following treatment of seed (Reynolds *et al.* 1957). In most of the early experimental work with the compound, little indication of a reduction of emergence of cotton seedlings was noted. In 1957, during an abnormally cool planting season, a serious reduction in emergence occurred in the San Joaquin Valley of California and in other areas of the United States where seed treated with phorate was planted. Preliminary investigations (Erwin & Reynolds 1958) indicated that the reduction was associated with a seedling disease induced by *Pythium* spp. In these studies a phorate-captan seed treatment induced a higher percentage of emergence than phorate-Ceresan. This finding has been consistent in several subsequent greenhouse tests. The following data are typical in unsterilized field soil at a low temperature (16° C.): emergence from phorate-captan was 80%, while that from phorate-Ceresan was 34%. In the same test, emergence from captan was 76%, and from Ceresan 86%. However, in later field experiments, emergence from seed treated with phorate-captan was not consistently as great as that from either Ceresan or captan (Erwin *et al.* 1959). No reason for this lack of agreement between greenhouse and field studies has been found.

Evidence is presented here which indicates that when the carrier of the active chemical in phorate, activated charcoal, is used alone, it reduces the emergence of Ceresan-treated seeds to some extent but not so greatly as phorate formulated on charcoal. The effects of dosage of phorate and age of treated seed on emergence as well as a comparison of the effects of phorate and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) on emergence are also presented.

METHODS AND MATERIALS.—The method of treatment of acid-delinted cotton seed (variety Acala 4-42) with phorate and fungicides has been previously reported (Erwin & Reynolds 1958, Erwin *et al.* 1959). The source of phorate was a proprietary formulation, Thimet 44D, which contained 44% active chemical and 56% activated charcoal. Treated seed was stored in screw-cap jars at 24° to 27° C. Seed was planted in 18×18×4-inch flats of soil at ½-inch depth. In most tests eight rows were planted in each flat with 50 seeds from each treatment distributed in two rows. To prevent possible diffusion of seed treatment chemicals to adjacent rows, each 2-row plot was separated from the adjacent one by means of a waterproof strip of insulating paper or metal inserted in

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[2] Associate Plant Pathologist, Department of Plant Pathology; Associate Entomologist, Department of Entomology; and Associate Biometrician, Biometrical Laboratory, respectively.

the soil. Treatments were randomized and replicated four or more times except where otherwise indicated, and each test was repeated at least twice and often 4 to 5 times. Soil moisture was not controlled, but the soil in the flats was watered routinely 24 hours before planting with no further irrigation until the plants emerged 6 days later. After planting, the flats were placed in a cold room at 16° to 18° C. for 6 days and moved to a greenhouse bench where air temperatures varied from 21° to 32° C. Emergence of seedlings was recorded every 2 or 3 days until completed. Data were analyzed statistically using the multiple range test of Duncan (1955).

Unless otherwise designated, the soil was a mixture of 3 parts granitic loam, 1 part peat moss, and 1 part silty sand. This mixture, designated Riverside soil, was naturally infested with two seedling disease pathogens, *Rhizoctonia solani* Kühn and a *Pythium* sp. Since the *Pythium* sp. could not be induced to form sexual bodies in culture, it was not identified although its mycelial growth and asexual sporulation appeared similar to that of *P. ultimum* Trow. In some tests the soil was rendered pathogen-free by steaming for 1 hour.

The following fungicides were used as seed treatments at the indicated rate per 100 pounds of acid-delinted cotton seeds: Ceresan 100 (ethyl mercury 2,3-dihydroxy propyl mercaptide, 3.1% and ethyl mercury acetate, 0.67%), 2 ounces; Ceresan 200 (double the concentration of active material in Ceresan 100), 1 ounce; and captan (N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, 75%), 2 ounces. Since both Ceresan 100 and 200 contain identical chemical ingredients at different concentrations, and since the dosage was equilibrated in different tests by applying the same amount of active ingredient to the seed, the numerical suffixes 100 and 200 are omitted.

RESULTS.—*Emergence of Treated Cotton Seed in Steamed and Unsteamed Soils.*—In steamed soil when cotton seed was treated with phorate or activated charcoal alone (equivalent to the amount used as a carrier of phorate in the proprietary compound), the emergence (95% to 98%) at soil temperatures of 16° and 21° C. was comparable to that from nontreated seed. As previously reported by Reynolds (1958) seedlings from phorate-treated seed showed necrotic areas on the cotyledonary leaves, primarily on the peripheral portions where systemic material accumulates at the end of the transpiration stream, but no other evidence of phytotoxicity.

In another experiment in naturally infested Riverside soil, a severe reduction in emergence occurred from seed treated with phorate-Ceresan. The percentages of emergence from the seed treatments were as follows: Ceresan, 60%; phorate-Ceresan, 7%; phorate, 0%; and no treatment, 0%. The same treatments in steam sterilized soil produced approximately 96% emergence. Averages from another series of four experiments with phorate-Ceresan showed an emergence of 83% in steamed soil but of only 64% in unsteamed soil. Numerous trials yielding similar results indicated that the phorate effect was probably associated with soil-borne pathogens.

In previous work a high percentage of phorate-treated seeds emerged in soil infested with *Rhizoctonia solani* Kühn but none emerged in soil infested with *Pythium ultimum*. (The fungus previously designated *P. debaryanum* Hesse has since been determined to be *P. ultimum*) (Erwin & Reynolds 1958).

To determine whether the emergence reduction associated with phorate-Ceresan seed treatment was due to *Pythium* spp., seed treatments were compared in lots of steamed Riverside soil artificially infested with each of several *Pythium* isolates (most of which appeared to be *P. ultimum*) from the San Joaquin Valley and from Riverside. Soil was infested with inoculum (test fungus grown on sterile oats) at a rate of 0.05% of the weight of the air-dry soil, incubated 3 weeks, and further diluted 1:1 with steamed soil. At this inoculum level all *Pythium* isolates induced a higher percentage of seedling loss from phorate-Ceresan than from Ceresan alone. For instance, in soil infested with the Riverside *Pythium* sp. the emergence of Ceresan-treated seed was reduced by the addition of phorate from 32% to 10%; in soil infested with a *P. ultimum* isolate from the San Joaquin Valley, from 90% to 32%; and with another isolate, from 95% to 47%. When inoculum levels of these fungi were higher, complete loss of seedlings from both treatments occurred. Naturally occurring pathogenic *Pythium* sp. appeared to cause a major part of the pre-emergence seedling loss associated with application of phorate to Ceresan-treated seed.

The Effect of Soil Temperature on the Emergence of Phorate-treated Seed.—Since reduction of soil temperatures to 16° to 18° C. or lower in the San Joaquin Valley has been commonly associated with an increase in incidence of seedling disease, the effects of seed treatment of cotton with phorate-Ceresan were compared at two temperatures, 16° C. at which cotton seed germinates slowly and 21° to 24° C. at which germination is more rapid.

The emergence-reducing effect of phorate was much greater at the lower soil temperature; for example, in one experiment the addition of phorate to Ceresan-treated seed reduced emergence at 16° C. from 98% to 69%, but at 21° to 24° C., from 98% to 82%. In a second experiment, emergence from Ceresan-treated seed at 16° C. was reduced from 97% to 68% by addition of phorate, but at 21° to 24° C., only slightly from 99% to 91%. Emergence from nontreated seed was 65% at 21° to 24° C. compared with only 16% at 16° C. At both temperatures the emergence from phorate-treated seed was comparable to that from nontreated seed. In a third experiment in which the soil was incubated at 16° for 11 days, a longer period than usual, no emergence occurred from phorate-Ceresan-treated seed compared with 21% emergence from Ceresan-treated seed, but at 21° to 24° C. for 4 days, 90% emergence was recorded for phorate-Ceresan and 98% for Ceresan-treated seed.

The first serious reduction in emergence of cotton due to phorate seed treatment in California was observed in the abnormally cool 1957 season. The absence of an obvious emergence-reducing effect of phorate in early tests with this compound (Reynolds *et al.* 1957) may have been due to the prevalence of high soil temperatures in field and greenhouse studies. Since cotton seedlings emerge most rapidly at temperatures of 30° C. or above (Arndt 1957), these experiments also support a concept developed by Leach (1947), namely, " . . . the ratio between the coefficient of velocity of seedling emergence

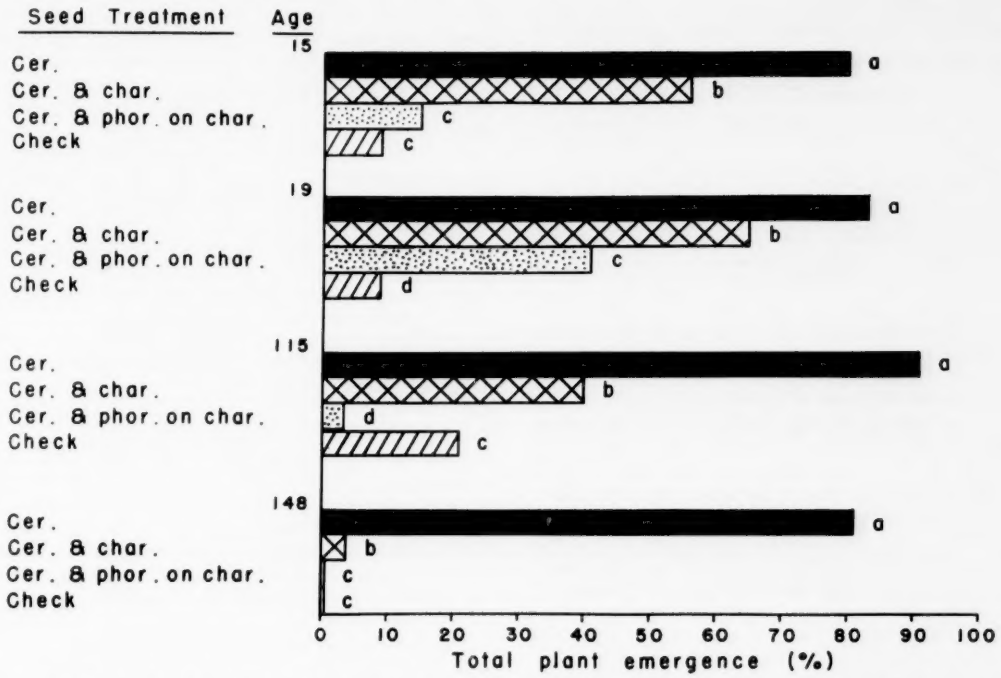

FIG. 1.—The effects of applying either activated charcoal (char) or phorate (phor), active chemical carried on activated charcoal, to Ceresan-treated cotton seed. Age indicates number of days between seed treatment and planting. Statistical populations for each experiment at the 99% level of confidence are designated by the letters a, b, c, and d.

and the growth rate of the pathogen is inversely related to the severity of infection."

Comparison of the Effects of Phorate-Ceresan and Activated Charcoal-Ceresan Seed Treatments on Emergence of Cotton.—To determine whether the active organic mercury components of Ceresan might be affected by the activated charcoal carrier of the active chemical in phorate, some seed was treated with an amount of charcoal equivalent to that in commercial phorate following the usual treatment of seed with Ceresan. Each test included four seed treatments: 1) Ceresan, 2) Ceresan plus activated charcoal, 3) Ceresan plus phorate (active chemical plus activated charcoal), and 4) no treatment. The average percentage of plants that emerged in naturally infested soil from each treatment in four experiments is shown graphically in figure 1. Since emergence from some treatments decreased with increasing age of treated seed, the age of the seed treatment at planting time is also shown.

The addition of phorate to Ceresan-treated seed reduced the emergence more than the addition of charcoal alone. This information indicated that both the active chemical phorate and its charcoal carrier play a part in reducing the emergence of Ceresan-treated seed. Since in other tests the addition of charcoal alone to untreated seed had no effect on emergence in steamed *Pythium*-infested, or *Rhizoctonia*-infested soil, the effect of charcoal alone on Ceresan-treated seed appeared to be due to an activated charcoal-fungicide interaction.

Since the active chemical in phorate is absorbed and translocated in the plant (Reynolds *et al.* 1957), the mechanism of its emergence-reducing effect may be physiological. If there is such a physiological effect, it appears to predispose the plant to *Pythium* seedling disease since no reduction in emergence was seen in soils freed of the fungus by steaming.

In another experiment, phorate was formulated on an absorptive silicon-base carrier. When it alone was added to Ceresan-treated seed, the emergence was the same as from Ceresan-treated seed (72%), but in combination with phorate emergence was reduced to 10%.

Effect of Phorate Dosage on Emergence.—To test the effect of dosage of phorate on emergence, the following seed treatments were prepared: no treatment, phorate (2 and 8 pounds per 100) applied to nontreated and Ceresan-treated seed, and Ceresan. In the first experiment, which was conducted in the field at Riverside, 200 seeds per treatment were planted in a row 50 feet long in a randomized block (6 replications).

Emergence of seedlings is shown in table 1. Emergence of Ceresan-treated seeds was reduced by the addition of phorate at 8 pounds per 100, but not when phorate was added at the rate of 2 pounds per 100.

Three subsequent experiments with the same lots of treated seeds were conducted in the greenhouse in naturally infested soil at low temperatures. The results (table 1) showed that 8 pounds of phorate per 100 pounds of Ceresan-treated seed reduced emergence more than 2

Table 1.—Effect of dosage of phorate (per 100 pounds of acid delinted seed) on reduction of emergence of cotton seedlings.

	Emergence (%)[a]					
	Seed Treatment					
Experiment	Ceresan	Phorate (2 lb.) plus Ceresan	Phorate (2 lb.)	Phorate (8 lb.) plus Ceresan	Phorate (8 lb.)	None
Field	85_b	87_b	67_a	73_a	64_a	68_a
Greenhouse	68_c	40_b	2_a	9_a	3_a	1_a

[a] The subscripts represent statistical populations at the 99% level of confidence, respectively for each experiment.

pounds. These data indicated that dosage of phorate may be a critical factor.

Effect of Storage Age of Treated Seed on Emergence.—The results of two series of experiments, summarized in figure 1 and table 2, suggested that emergence from phorate-Ceresan-treated seed was reduced with increasing age of the seed treatment. Since each experiment in which seed of a different age was used was conducted at a different time, another experiment was conducted in which the seeds were treated with phorate-Ceresan at 1, 10, and 17 days before planting in naturally infested soil. Emergence was reduced from 74% for the 1-day-old treatment to 47% to 54% for the 10- and 17-day-old treatments. In a second experiment emergence from seed treated with phorate-Ceresan 243 days before planting was 0%, but from seed treated the same day as planting emergence was 90% compared with 92% from Ceresan and 33% from nontreated seed. No effect of age of Ceresan treatment on emergence was noted. Reduction in emergence due to the age effect on phorate-Ceresan-treated seed was significantly different from that for Ceresan at the 99% level of confidence in both experiments.

To determine the possible effect of moisture from the methyl cellulose sticker solution (used at 3 quarts per 100 pounds of seeds), seeds treated with phorate-Ceresan were stored for 115 days in manila bags lined with porous paper and in capped jars. In naturally infested soil, reduction in emergence due to phorate was approximately the same with both methods of storage. The mechanism of the aging effect under these conditions did not appear evident.

Comparison of Phorate and Di-Syston as Seed Treatments.—Since Di-Syston is chemically quite similar to phorate, it was of interest to compare the two compounds as seed treatments, alone and in combination with Ceresan. Dosage of Di-Syston (50% active chemical and 50% activated carbon) was equilibrated to that of phorate. The results are summarized in table 2. A greater emergence was recorded for seed treated with Di-Syston-Ceresan than with phorate-Ceresan. Although emergence from seeds treated with Di-Syston-Ceresan was relatively high, emergence was delayed for at least 1 to 2 days and reduction in plant size was evident. For instance, 15 days after planting in steamed soil when 130-day-old-seed was used, the average height of seedlings from the Di-Syston-Ceresan treatment was only 7 cm. compared with 10 to 12 cm. from other seed treatments of the same age.

Table 2.—Comparison of phorate and Di-Syston seed treatments in combination with Ceresan.

	Emergence (%)[a]					
	Seed Treatment					
Age of Seed Treatment (days)	Ceresan	Phorate	Phorate plus Ceresan	Di-Syston	Di-Syston plus Ceresan	None
5–28[b]	95_d	32_b	64_c	66_c	84_d	19_a
130	51_b	2_a	1_a	2_a	54_b	2_a

[a] The subscripts represent statistical populations at the 99% level of confidence respectively for each experiment.

[b] Data are summarized from four 2-replicate experiments in which age of seed treatment was not statistically a factor.

Discussion.—It would be of basic interest to know the mechanism of the apparent predisposition effect of phorate on cotton seed in soil artificially or naturally infested with *Pythium*. Hacskaylo (1957) showed that in young cotton plants treated with phorate (10 p.p.m. of active chemical) in a nutrient solution, reducing sugars, sucrose, and starch were increased while both soluble and protein nitrogen were decreased. In leaves both chloroform soluble (lipid) and insoluble phosphorus were increased. He also noted a temporary wilting of all plants treated with phorate although adequate moisture was present. When more biochemical data are available, perhaps the nature of the increase in susceptibility of cotton seedlings to *Pythium* may be explained. Such information may also be of interest for studies of resistance and susceptibility of seedlings to "damping-off" pathogens.

Considering the rapid increase in use of systemic insecticides, a great need exists for more information concerning their biochemical effect on the plant's physiology which may, in turn, affect its susceptibility to disease.

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Determining the Appropriate Transformation of Data from Insect Control Experiments for Use in the Analysis of Variance[1]

H. Y. Forsythe, Jr., and George G. Gyrisco, *Cornell University, Ithaca, N. Y.*

Abstract

The procedure used to determine the appropriate transformation of insect control data consisted of testing the untransformed data for fulfillment of the basic assumptions underlying the analysis of variance procedure and its related tests of significance. The data were tested two more times, once when the data were transformed to the square-root scale and once when transformed to the log scale.

A nonparametric, rank-correlation coefficient was computed for the ranges and means of each set of data for evidence of normality. A nonsignificant coefficient was an indication that the ranges and means were independent of each other, thus indicating normality of the data. After normality had been established, Bliss and Calhoun's modification to Hartley's test for heterogeneity of variance and Tukey's test for nonadditivity were computed for each set of data. A set of data was considered appropriate for use in the analysis of variance when the data fulfilled the basic assumptions of the analysis procedure.

For illustrative purposes this procedure was used on two control experiments, one on the potato leafhopper (*Empoasca fabae* (Harris)) and one on the alfalfa snout beetle (*Brachyrhinus ligustici* (L.)) larva. The leafhopper data transformed both to the square-root and log scales were found to fulfill the assumptions while a log transformation appeared to be the best scale to use for the snout beetle data.

In the statistical analysis of an entomological control experiment the analysis of variance procedure and a related test of significance often are used to determine significant treatments. However, these procedures are based on four assumptions which the entomologist must consider before his statistical inferences are considered valid. The four basic assumptions underlying the analysis of variance procedure and the related tests of significance are: (1) the treatment effects and the environmental effects must be additive, (2) the experimental errors must have a common variance, (3) the experimental errors must all be independent, and (4) the experimental errors should be normally distributed. These assumptions are summarized in the linear model for the analysis of a randomized complete block experiment—the experimental design commonly used in entomological control experiments—as:

$X_{ij} = u + T_i + P_j + e_{ij}$; e_{ij} is $NI(O,\sigma)$ where: X_{ij} = observed value for the i^{th} treatment and the j^{th} replicate, u = population mean, T_i = effect of the i^{th} treatment, P_j = effect of the j^{th} replicate, and e_{ij} = experimental error of the observed value.

The degree to which the assumptions are met apparently determines the validity of the inferences drawn from the analysis of variance. Slight departures from the assumptions would imply that significance levels must be considered approximate rather than exact. Cochran (1947) has stated that the failure to meet any of the four basic assumptions will affect the power and sensitivity of the F- and t-tests, as well as the significance levels. In the case of nonadditivity, the error variance would be enlarged due to the effects being multiplicative instead of additive and to the interaction between treatment and environmental effects (Tukey 1949, Kempthorne 1952). If the error mean square were calculated when heterogeneous treatment variances were present in the data, the error mean square would just be an average of the individual variances and not really applicable to testing any of the treatment comparisons (Cochran 1938, Bartlett 1947). Nonindependence and non-normality of the data may also bias the estimate of the error mean square, with similar impairment of the tests related to the analysis of variance (Cochran 1947).

Often these assumptions are overlooked or ignored by entomologists. Even when an entomologist realizes that his data may violate an assumption, he may be at a loss to known how to treat his data. There are various methods which can be used to obtain an improved analysis, but perhaps the most generally accepted and useful method to treat many kinds of data is to transform the original data to a scale in which all assumptions are fulfilled. The most commonly used transformations in insect count data are the square-root and the logarithmic transformations. There are a number of other possible transformations, of which Bartlett (1947) gives a list in his article.

There are a number of procedures which may be used to determine an appropriate transformation. Some transformations have been determined mainly on the basis of the insect distribution form (Beall 1942, Anscombe 1949). Often, however, the entomologist does not know the distribution form for the particular insect with which he is concerned. If the distribution form is known, it is highly possible that the distribution pattern may vary because of the effects of certain insecticides or due to a different number of net sweeps taken per plot. It is also possible to get an idea of an appropriate transformation by plotting the variances and the standard deviations against the treatment means. This procedure simplifies computations in determining an appropriate transformation, but it may be difficult to determine definitely which transformation is more appropriate. Another procedure the entomologist can use is to run certain tests to determine fulfillment of the assumptions against two analyses of variance, one consisting of untransformed data and one of transformed data.

The present report is intended to outline a possible simplified procedure which entomologists may use to determine the appropriate transformation scale and at the same time can be confident that the data fit the assumptions underlying the analysis of variance procedure. Two

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types of insect control experiments are presented here for illustrative purposes and the appropriate transformation scale is determined for each.

STATISTICAL METHODS AND PROCEDURE.—Tukey (1949) has devised a test for determining nonadditivity, and Hartley (1950) has devised a test to detect heterogeneity of variances. However, Tukey's test is based on normal or infinite model theory and Hartley's test is sensitive to normality as well as to heterogeneity of variances. Therefore it is essential to have the original data normally distributed, or nearly so, before running these tests on the data.

In a normal distribution the variability among the observations is independent of its mean (Bartlett 1947, Bliss & Calhoun 1954). To determine whether the data can be considered normal, a nonparametric, rank-correlation coefficient (Hotelling & Pabst 1936, Dixon & Massey 1951) was computed for the ranges and means of each set of data. This coefficient was used to test the hypothesis that the ranges and means were independent of each other, thus indicating normality of the data. An advantage of this rank-correlation procedure is that no assumption need be made concerning the form of distribution of the data.

After normality of each set of data had been established, a modification to Hartley's test (Bliss & Calhoun 1954) and Tukey's test were then computed. This procedure was first carried out on the untransformed data. If the data were found to be non-normal, then transformations of the original counts were made to the square-root and log scales, and the same procedure was carried out on each scale. The set of data which can be confidently used in the analysis of variance procedure is the set which fulfills the assumptions the best.

The assumption of independence of the experimental errors is usually satisfied by the physical act of randomization of the treatments in the field—a practice customarily followed in the setting up of a randomized complete block experiment (Cochran 1947).

The range was used in the computations of the rank-correlation coefficient and of Bliss and Calhoun's test for heterogeneity. Bliss & Calhoun (1954) and Snedecor (1956) have recognized the use of the range as a fairly efficient measure of the variability of the observations in a set of data. The range was therefore used instead of the variance or standard deviation, primarily because of its ease of computation in these preliminary tests.

EXPERIMENTAL DATA CONSIDERED.—Two entomological experiments were selected from actual field-control work done in the Forage and Cereal Insect Investigations Section of the Department of Entomology and Limnology at Cornell University. Two forage insect pests were considered: the potato leafhopper, *Empoasca fabae* (Harris), and the alfalfa snout beetle, *Brachyrhinus ligustici* (L.).

A randomized complete block design was set up in an alfalfa field in an insecticide-control study of the potato leafhopper, and emulsifiable insecticides were sprayed onto the plots in mid-summer. Later in the summer 30 net sweeps were taken in each plot. The adults and nymphs in each set of sweeps were counted. See table 1 for the number of leafhoppers found. This table also shows the means and ranges of data that are untransformed, and of data transformed to a square-root or log scale.

In an insecticide control study of the alfalfa snout beetle, a randomized complete block design was set up in an alfalfa field, and granulated insecticides were applied to the plots in the spring. In the fall six 1-square-foot soil samples were dug per plot. The larvae in each sample were counted. See table 2 for the number of larvae found. This table also shows the means and ranges of the data on untransformed and transformed scales.

DISCUSSION OF RESULTS.—The data in table 3 summarize the results obtained in the application of the proposed procedure for determining the appropriate transformation in two entomological control experiments.

A significant correlation between the mean and range of counts in the potato leafhopper control experiment, as determined by the rank correlation coefficient, was found in the set of untransformed data. This significant rank correlation coefficient indicated that the range was not independent of the mean, and it was considered as evidence of non-normality. Therefore, the analysis of variance procedure could not be applied to this set of data with confidence. The transformed sets of data, which were apparently normal, were tested for heterogeneity of variance and nonadditivity. No significant results were found, thus indicating that both these sets had fulfilled

Table 1.—Untransformed numbers of potato leafhoppers taken in 30 sweeps per plot.

	Treatments								
Replicates	1	2	3	4	5	6	7	8	9
I	1	1	7	0	5	4	2	5	25
II	0	2	1	0	6	6	2	1	15
III	0	8	1	1	7	4	0	2	9
Untransformed scale: $y=X$									
Mean:	0.3	3.7	3.0	0.3	6.0	4.7	1.3	2.7	16.3
Range:	1	7	6	1	2	2	2	4	16
Transformed scale: $y=(X+1.0)^{1/2}$									
Mean:	1.14	2.05	1.88	1.14	2.64	2.38	1.49	1.86	4.09
Range:	0.41	1.59	1.42	0.41	0.38	0.41	0.73	1.04	1.94
Transformed scale: $y=\log(X+1.0)$									
Mean:	0.100	0.577	0.502	0.100	0.842	0.748	0.318	0.519	1.206
Range:	0.301	0.653	0.602	0.301	0.125	0.146	0.477	0.477	0.415

Table 2.—Untransformed numbers of alfalfa snout beetle larvae taken in six 1-square-foot soil samples per plot.

REPLICATES	TREATMENTS						
	1	2	3	4	5	6	7
I	1	1	1	26	1	3	23
II	1	2	6	58	5	13	12
III	0	1	3	17	0	43	87
IV	1	7	2	10	4	14	13
V	0	1	2	9	1	7	11
Untransformed scale: $y=X$							
Mean:	0.6	2.4	2.8	24.0	2.2	16.0	29.2
Range:	1	6	5	49	5	40	76
Transformed scale: $y=(X+1.0)^{1/2}$							
Mean:	1.25	1.76	1.90	4.72	1.70	3.81	5.02
Range:	0.41	1.42	1.24	4.52	1.45	4.63	5.92
Transformed scale: $y=\log(X+1.0)$							
Mean:	0.181	0.457	0.540	1.298	0.416	1.094	1.333
Range:	0.301	0.602	0.544	0.771	0.778	1.042	0.865

the assumptions underlying the analysis of variance procedure.

The correlation between the mean and range of counts of the alfalfa snout beetle larvae was found to be significant at the 10% level for the untransformed data and for the set of data transformed to the square-root scale. Since the range was apparently not independent of the mean, these sets of data would have to be considered nonnormal, and the analysis of variance procedure would be inappropriate. When the remaining set of data which were transformed to the log scale was tested for heterogeneity of variance and nonadditivity, no significant results were found. Therefore, it appears that only the log data could be analyzed with confidence in the analysis of variance procedure.

On the basis of these results it would seem that before a statistical analysis is made, potato leafhopper control data of the type mentioned should be transformed to the square-root or log scale, while alfalfa snout beetle data should be transformed to the log scale. However, a word of caution must be given at this point concerning the use of a particular transformation scale for similar leafhopper or snout beetle control experiments on the basis of transformation results obtained in only one experiment. In general, a larger number of experiments must be considered and analyzed for any single insect species before conclusions can be drawn as to the appropriate transformation scale for that species. Even after a generalization is made, some caution must be exercised concerning the use of a particular transformation.

Table 3.—Results of study on appropriate transformations to use in the analysis of variance for one set of potato leafhopper data and for one set of alfalfa snout beetle data.

SCALE OF DATA	TEST FOR NON-NORMALITY[a] r_s	TEST FOR HETEROGENEITY OF VARIANCES[b] $F_{max.}$	TEST FOR NON-ADDITIVITY[c] F_{NA}
	Potato Leafhopper Data		
$y=X$	$+0.683$[d]	—	—
$y=(X+1.0)^{1/2}$	$+0.288$[e]	26.11[e]	1.48[e]
$y=\log(X+1.0)$	-0.146[e]	27.25[e]	<1.00[e]
	Alfalfa Snout Beetle Data		
$y=X$	$+0.938$[d]	—	—
$y=(X+1.0)^{1/2}$	$+0.821$[d]	—	—
$y=\log(X+1.0)$	$+0.607$[e]	11.97[e]	<1.00[e]

[a] Rank correlation coefficient,

$$r_s = 1 - \frac{6(\Sigma d_i^2)}{N(N^2-1)},$$

where N = number of ranks and d_i = difference between ranks of the range and mean pairs. Tested at 10% level.

[b] Bliss and Calhoun's $F_{m.x} = (R_{max.}/R_{min.})^2$, where R = range. Tested at 5% level.

[c] Tukey's $F_{NA} = MS_{NA}/(MS_{Error} - MS_{NA})$, where MS_{NA} = mean square for nonadditivity. Tested at 5% level.

[d] Significant at 10% level.

[e] Not significant.

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Barn Fogging as a Fly Control Method[1]

G. R. DeFoliart and J. L. Eschle,[2] *Department of Entomology, University of Wisconsin, Madison*

Abstract

Compressed-air barn foggers utilizing a fogging solution containing 0.1% pyrethrins and 1.0% piperonyl butoxide gave adequate control of house flies (*Musca domestica* L.) in dairy barns and horn flies (*Haematobia irritans* (L.)) on cows. The mortality rate was inadequate if the pyrethrins content was lowered and repellents substituted in the formulation. Fogging was two to four times more costly than a program utilizing residual barn spraying for house fly control and methoxychlor powder treatment of cows for horn flies. Fogging did not provide pasture protection against stable flies (*Stomoxys calcitrans* (L.)) or horse flies (Tabanidae).

A fogging method developed for fly control in dairy barns was described by Fisher (1955). As performance data were not included, data obtained during 1960 comparing fogging with residual spraying of barns are presented here. Most of the control procedures were done by the cooperating farmers and the results represent the effectiveness of the methods in the hands of the farmers themselves.

Methods.—Tests were conducted on 22 dairy farms, 12 in north-central Wisconsin (Taylor County) and 10 in south-central Wisconsin (Dane County). Practical control was more difficult to obtain in Dane County than in Taylor County and necessitates separate consideration of some of the results in the two areas. Farms are closer together, herds average somewhat larger and the climate is slightly warmer in Dane County, all of which might increase population pressure on the control methods, but it is not readily apparent why there was such a great difference in the two localities. Half of the barns were fogged and the others were sprayed. The cooperating farmers fogged their barns at least once daily and some twice daily. The average amount of solution used per day was figured at the end of the season. In barns that were sprayed, the farmers did their own spraying in Taylor County whereas project personnel did the spraying in Dane County. Herds milked in sprayed barns were hand-sprayed by the owners, usually every other day, but the schedule varied.

House fly (*Musca domestica* L.) counts in the barns were made with a Scudder fly grill placed at four locations where flies appeared most numerous. Control was considered satisfactory when counts from the four placings totaled fewer than 50 or 60 flies. Untreated barns were not available, but observations indicated that grill counts would have exceeded 200 in Dane County barns and at least 100 in Taylor County barns in the absence of control measures. The counts frequently would have been much higher since counts of more than 600 were obtained in both areas in barns where control measures were in use. A count of 50 to 60 flies evolved as the upper limit of practical control because when such counts were obtained, flies obviously bordered on over-abundance. Once flies reached this level following spraying, they increased rapidly to a level that resulted in counts of 200 or more. The lower figure (50) was strictly adhered to in Taylor County where control was easier, while more latitude was allowed in Dane County. Counts were made approximately at weekly intervals.

Table 1.—Grill counts of house flies in dairy barns that were sprayed with a residual insecticide in Taylor County, Wisconsin. 1960.

Insecticide		Date Applied	Fly Grill Counts—After Weeks Shown[a]						
			1	2	3	4	5	6	7
Diazinon,	w.p.	7/29	0	0	—	4	—	—	11→
	e.c.	7/9	—	4→					
	e.c.	7/26	2→						
	e.c.	8/9	—	3	—	—	0→		
Ronnel,	w.p.	6/14	2	1	—	—	6	34→	
	w.p.	7/21	17→						
	w.p.	8/5	33	—	33→				
	w.p.	9/1	—	5→					
	e.c.	8/24	—	—	14→				
Dimethoate,	w.p.	7/7	—	6	—	15	34→		
	w.p.	8/11	—	2	—	—	1→		
	e.c.[b]	7/1	—	2	8	3	—	17	—

[a] An arrow indicates control extended well beyond the date of the last count.
[b] 8th week, 13; 9th week, 18; 11th week, 181.

Counts of horn flies (*Haematobia irritans* (L.)), stable flies (*Stomoxys calcitrans* (L.)) and horse flies (Tabanidae) were taken at the same time on corresponding herds, all of the flies being counted on 10 animals in the pasture. Untreated control animals were not counted regularly. Past experience and counts at various times on untreated heifers during 1960 indicated that horn flies exceeded 50 per animal throughout the summer. Dusted herds were used as controls for stable flies and tabanids as it is generally recognized that dusting the backs of cattle with methoxychlor at 3-week intervals does not appreciably reduce the number of these flies.

The following formulations were used in foggers. The one referred to hereafter as 0.1% pyrethrins consisted of 0.1% pyrethrins, 1.0% piperonyl butoxide, 0.8% petroleum distillate, and 98.1% mineral seal oil and deodorized kerosene (50:50). One or the other of three repellents, MGK Repellent 11® (2,3,4,5-bis(Δ2-butylene)-tetrahydrofurfural), MGK Repellent 326® (di-*n*-propyl isocinchomeronate) or MGK Repellent 1207® (3-chloropropyl *n*-octyl sulfoxide) were incorporated at the level of 0.4% in a formulation otherwise consisting of 0.075% pyrethrins, 0.15% piperonyl butoxide, 0.25% N-octyl bicycloheptene dicarboximide (MGK 264), and 99.125% petroleum distillate. These are referred to hereafter as R-11, R-326, and R-1207, respectively.

Results and Discussion.—Fogging and spraying for house fly control in dairy barns in Taylor County shows that barn spraying with residual insecticides was generally superior to fogging (tables 1, 2). On only one occasion did the grill count exceed 50 in a sprayed barn, and that was 76 days after application, whereas fogged barns showed poor control on 13 occasions. Excellent control

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Table 2.—Grill counts of house flies in dairy barns that were fogged in Taylor County, Wisconsin. 1960.

Formulation	Fluid Oz./Linear Ft./Day	Fly Grill Counts 7/2	7/15	7/21	7/29	8/3	8/10	8/24	9/3	9/14
R-11 (Fa)[a]	0.20	—	15	44	103	—	61	66	63	55
R-11 (Da)	.28	27	—	17	437	15	12	625	—	31
R-1207 (Do)	.37	3	—	3	—	5	50	9	—	10
R-1207 (We)	.20	5	—	30	203	17	56	59	—	59
Pyrethrins (Me)	.27	14	—	—	0	—	—	38	—	9
Pyrethrins (Bo)	.12	—	11	110	16	—	24	5	3	5

[a] Barn identification letters in parentheses.

was obtained, without exception, by spraying with Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate), ronnel or dimethoate, and despite the fact that in most cases only 3 gallons of spray containing 1.0% of the insecticide were used to treat barns 60 to 80 feet long. The farmers sometimes re-sprayed when there were far too few flies in the barn to warrant it. The longest completed test was with dimethoate which maintained control for nearly 11 weeks. Under the conditions in 1960, it appears that a single spraying with any of the three insecticides should provide at least 6 weeks of control, and that a maximum of two sprayings are necessary during the season. Three of the six farmers sprayed only once.

Of the three formulations used in foggers, 0.1% pyrethrins was the most efficient, being the only one that gave practical control with a practical volume of solution. R-11 and R-1207 each did well when relatively large volumes (0.28–0.37 fl. oz. per linear foot) were used, but at smaller volumes (0.20 fl. oz. per linear foot) they did not perform so well.

Neither fogging or spraying gave as good control in Dane County (tables 3 and 4) as in Taylor County. Only 4 to 5 weeks of satisfactory control was obtained from applications of 6 to 8 gallons of 1.0% spray of Diazinon or dimethoate. Flies were not held at as low levels as they were in Taylor County despite the application of more spray material. Frequently, less than 2 weeks of control was obtained with ronnel (Korlan®) and Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate). In foggers (table 4), 0.1% pyrethrins gave the best control and required the lowest volume output. In one of two barns R-1207 was satisfactory, and 0.5% Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) was effective in one short test (not shown) near the season's end.

Table 3.—Grill counts of house flies in dairy barns tha were sprayed with a residual insecticide in Dane County, Wisconsin. 1960.

Insecticide		Date Applied	Fly Grill Counts After Weeks Shown[a] 1	2	3	4	5
Diazinon,	w.p.	7/1	12	2	37→		
	w.p.	7/22	—	40	158*	46	235*
	e.c.	7/1	4	37	62		
	e.c.	7/19	90[b]	91[b]	57	47	300*
	e.c.	8/31	124*	75			
Dimethoate,	w.p.	7/19	6	60	29	64*	212*
	e.c.	8/30	72*	9	—	24→	
Ronnel,	w.p.	6/24	—	23	19	57	
	w.p.	7/21	10	393*	212*		
	w.p.	8/17	9	42	213*	276*	
	w.p.	8/30	107*	118*			
	e.c.	7/19	102*	277*			
	e.c.	8/17	50	74*	136*		
	e.c.	8/17	91*	73*	227*		
Bayer 29493,	w.p.	7/8	—	151*			
		7/22	—	95*	83*	153*	

[a] Arrow indicates control longer than shown although no later count was made. Asterisk indicates the count too high for good control.

[b] One unduly high count obtained by placing the grill over a milk pan for cats made this figure not representative of the number of flies in the barn.

All formulations in barn foggers provided excellent horn fly control in Taylor County; herds milked in fogged barns needed no additional control measures for this pest. Counts were mostly below five per animal and usually fewer than one per animal (table 5A).

In Dane County, fogging with 0.1% pyrethrins resulted in only 1.8 horn flies per cow. In the other fogged herds, they averaged 5.4 to 12.5 per cow (table 5B).

Table 4.—Grill counts of house flies in dairy barns that were fogged in Dane County, Wisconsin. 1960.

Formulation	Fluid Oz./Linear Ft./Day	Fly Grill Counts[b] 7/5	7/11	7/19	7/26	8/3	8/9	8/16	8/23	8/30
R-11 (Mu)[a]	.51	26	—	—	71					
R-11 (Pe)	—	34	160*	131*						
R-326 (Mu)	.30	—	—	—	—	106*	132*	115*	250*	347*
R-1207 (St)	.28	112*	80*	76*	214*	621*	118*	134*	55*	282*
R-1207 (Ak)	.21	5	33	30	124*	64	54	54	87*	222*
Pyrethrins (Sw)	.16	36	34	60	41	138*	96*	11	61	19

[a] Barn identification letters given in parenthesis.

[b] Asterisk indicates count was too high for good control.

Table 5.—Fly counts on insecticide-treated dairy cattle in Taylor and Dane Counties, Wisconsin.

Method	Formulation	Average No. Flies per Cow		
		Horn-	Stable-	Horse-
	A. Taylor County			
Fog	R-11 (Fa)[a]	0.8	3.9	1.0
	R-11 (Da)	1.0	12.6	3.5
	R-1207 (Do)	5.6	1.4	0.5
	R-1207 (We)	0.4	9.7	2.5
	Pyrethrins (Me)	2.2	2.8	1.5
	Pyrethrins (Bo)	0.4	5.3	2.2
	Average	1.7	7.0	1.9
Hand-spray	R-11 (We)	5.9	2.0	0.3
	R-1207 (Tk)	4.4	3.1	1.0
	R-326 (Pe)	4.3	2.5	1.4
	Pyrethrins (Gr)	0.4	0.9	0.6
	Average	3.8	2.1	0.8
Dust	Methoxychlor (Pe)	1.9	1.6	0.3
	Sevin®[b] (Zu)	1.5	3.4	1.1
	Average	1.7	2.5	0.7
	B. Dane County			
Fog	R-1207 (St)	8.9	3.9	0.3
	R-1207 (Ak)	12.5	3.2	0
	R-326 (Mu)	5.4	9.9	—
	Pyrethrins (Sw)	1.8	13.3	0.3
	Average	7.2	7.6	0.2
Hand-spray	R-11 (Pe)	1.9	9.7	—
	R-11 (OM)	11.2	6.6	0.2
	R-1207 (Zi)	10.6	4.9	0.2
	Pyrethrins (Gi)	4.1	6.4	0.1
	Average	7.0	6.9	0.2
Dust	Methoxychlor (Bu)	4.2	4.1	0.2
	Methoxychlor (Zi)	0.4	8.1	—
	Average	2.3	6.1	0.2
	Malathion (Zi)	26.4	3.1	0.1

[a] Barn identification letters given in parenthesis.
[b] 1-naphthyl *N*-methylcarbamate.

Data on stable flies and horse flies (table 5) indicate no pasture protection of cows that were fogged. Hand-spraying, as done by these farmers, apparently provided protection in only one herd. Herds dusted for horn flies were considered suitable as controls for stable fly and horse fly counts. There was an average of only 2.5 stable flies and 0.7 horse flies on two dusted herds in Taylor County (table 5A) compared with an average of 7.0 stable flies and 1.9 horse flies on fogged herds and 2.1 stable flies and 0.8 horse flies on hand-sprayed herds. In one case (Herd Gr) hand-spraying with 0.1% pyrethrins evidently was responsible for reducing the number of these flies on cattle at pasture. Not only were stable fly and tabanid counts low, but there were only 0.4 horn flies per cow. Low natural populations are probably responsible for the other low averages obtained, *i.e.*, herd Do with 1.4 and 0.5 and herd We 2.0 and 0.3 stable flies and tabanids, respectively (table 5A). Horn flies were more than five per cow in both herds, indicating that the insecticide applied was not responsible for the low stable fly and tabanid counts obtained. That the fogging method does not offer pasture protection from stable flies and tabanids is emphasized by the fact that the number of these flies remained high even though the volume of solution fogged exceeded in every case the generally recommended one fluid ounce per 10 linear feet of barn. Also, when the data were broken down according to whether counts were made less than 12 hours or more than 12 hours after last fogging, no pattern was evident suggesting any protection from fogging. Results in Dane County (table 5B) against stable flies were similar to those in Taylor County. The methoxychlor-dusted herds used as controls averaged 6.1 stable flies and fogged and hand-sprayed herds averaged 7.6 and 6.9, respectively. The horse fly population was too low for evaluation of results.

In both areas, applying more insecticide might have caused a greater reduction of flies on hand-sprayed herds. Application rates averaged near 0.5 fluid ounce per cow on most farms on a daily basis.

The results obtained lead to the following conclusions:
1) Fogging, regardless of the formulation used, does not provide pasture protection against stable flies or horse flies. The maximum amounts of toxicant or repellent that could possibly be deposited on the cows by dispensing one fluid ounce per 10 linear feet of barn or any higher reasonable amount undoubtedly fall well below the level needed to achieve any effect on these flies. Hence, there would appear to be no benefits derivable from incorporating repellents such as R-11, R-1207, R-326, Tabutrex® (dibutyl succinate) or butoxy polypropylene glycol in formulations intended primarily for use in foggers.
2) Fogging gives adequate control of horn flies. This is evidently the result of continuous pressure of the toxicant action of the pyrethrins against the horn fly population, eventually reducing it to a low level similar to that resulting from spraying with a chlorinated hydrocarbon insecticide.
3) Fogging controls house flies in the barn if, in the case of formulations containing pyrethrins as the toxic agent, the pyrethrins content is maintained near 0.1%. The method is ineffective when repellents are substituted and the pyrethrins content reduced below 0.1%.
4) Fogging is an expensive method compared with residual spraying of barns. Under the conditions of the tests, fogging was two to four times more costly than a combination of residual barn spraying and herd dusting. The typical barn in these tests was 80 to 90 feet long. Fogging such a barn with 12 fluid ounces of solution daily for a 90-day season would require about 8.4 gallons. At a cost of $2.75 per gallon and assuming 30 cows milked in such a barn, the cost per cow for fly control in the barn and horn fly control on the cows averaged 77¢.

Farmers in Taylor County obtained control in barns of the above size by spraying an average of two times with 3 gallons of water containing 1.0% of insecticide. Based on the price of 25% Diazinon wettable powder, $1.50 per pound, their cost of fly control in the barn amounted to $3.00 or 10¢ per cow. To obtain horn fly control and make the benefits included the same as those for fogging,

four applications of methoxychlor powder would cost an additional 8¢ or 18¢ per cow. This compares with 77¢ for fogging. In Dane County, three barn applications were needed and about 6 gallons of spray per application so that cost of fly control in the barn amounted to about $9.00. Five dustings for horn fly control were necessary at 10¢ per cow, so that the total cost for fly control in the barn and horn fly control on the cows amounted to about 40¢ per cow compared with 77¢ for fogging. With both methods, any costs incurred for stable fly or horse fly control by hand-spraying or other means would be extra.

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Residual Activity of Organophosphorus Insecticides in Soil as Tested against the Eye Gnat, *Hippelates collusor*[1]

Mir S. Mulla, George P. Georghiou, and Harry W. Cramer,[2]
University of California Citrus Experiment Station, Riverside

Abstract

The long-term activity of 20 organophosphorus insecticides in soil was studied against *Hippelates collusor* (Townsend), the predominant species of eye gnats in the southwestern United States. Soil lots treated with three dosages of each material were stored in a glasshouse maintained at 88° ± 2° F. and bioassayed against the eye gnat at from 2- to 8-month intervals of storage.

Trithion®, Diazinon®, Bayer 25141, Co-Ral®, ethion, GC-4072, and Ethyl Guthion® at the intermediate or high dosage levels manifested appreciable residual activity against the gnat after 6 to 8 months of storage. Bayer 22408, phorate, American Cyanamid Experimental Nematocide 18133, GC-3583, and GC-3582 showed a great deal of biological activity at the intermediate or high dosage levels after 2 months of storage, but were not active at later intervals of testing.

The other materials, Methyl Trithion®, Bayer 29493, Guthion®, Di-Syston®, ronnel, dimethoate, parathion, and Bayer 22684 did not, even at the highest dosage levels, show any appreciable residual activity when they were tested 2 months after storage.

Chemical Definitions of Proprietary Materials

American Cyanamid Experimental Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate)
Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate)
Bayer 22684 (*O*-methyl-*O*-(2-chloroethyl)-*O*-(2-dichlorovinyl) phosphate)
Bayer 25141 (*O,O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)
Co-Ral (*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7yl phosphorothioate)
Diazinon (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate)
Di-Syston (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)
Ethyl Guthion (*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate)
GC-3582 (*O,O*-diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate)
GC-3583 (*O,O*-diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate)
GC-4072 (*O,O*-diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate)
Guthion (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl) phosphorodithioate)
Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate)
Trithion (*O,O*-diethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate)

Hippelates eye gnats breed predominantly in friable irrigated soils. Tillage or other forms of soil surface disturbances are conducive to increased breeding of these pests of animals and man. In the desert and coastal regions of California where belts of loose soil are common, intensive farming operations—including frequent irrigations and tillage—result in the production of immense numbers of eye gnats. The gnats in these areas constitute a constant source of annoyance to man and domesticated animals.

One possible approach toward abating these pests is through the incorporation of residual insecticides into the soil where the gnats are breeding. A good number of the residual organochlorine insecticides were evaluated in the laboratory (Mulla 1960a). The strain of *H. collusor* from the Coachella Valley was found to be highly resistant to BHC and cyclodiene insecticides compared with a strain collected in Orange County (Mulla 1960, unpublished data). Susceptibility of Coachella Valley strain to DDT was slightly lower than that of Orange County strain; no information was obtained on the susceptibility of the latter strain to Shell Development Company Compound SD-4402.[3] As a result of these studies, only DDT and Compound SD-4402 merited field investigations in the Coachella Valley.

Field investigations on the effectiveness of various organochlorine insecticides (SD-4402, DDT, dieldrin,

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[2] The assistance of Ronald B. Winslow and James Mayabb of the Citrus Experiment Station during the course of these studies is duly acknowledged.

[3] Proprietary materials are identified by their respective chemical definitions preceding "References Cited."

heptachlor, toxaphene and aldrin) demonstrated the effectiveness of DDT and SD-4402 against the gnats (Mulla 1961, Mulla *et al.* 1960). DDT at the rate of 14 pounds per acre yielded good control of the gnats for three breeding seasons. In 1958 the Coachella Valley Mosquito Abatement District, using 15 pounds of the toxicant per acre, initiated application of DDT to tilled farms. Approximately 10,000 to 12,000 acres were treated each year. However, late in 1960 there were indications that the susceptibility to DDT of *H. collusor* from the Indio area in the Coachella Valley had decreased compared with the susceptibility level of a strain from the same area before the large-scale DDT treatments were initiated (Mulla 1960, unpublished data). A four-fold tolerance to DDT at the LD_{50} and LD_{90} levels was demonstrated for the population tested in the latter part of 1960.

The development in eye gnats of tolerance to DDT and other organochlorine insecticides prompted the current studies on evaluating organophosphorus insecticides against the eye gnats. Most of these compounds were highly effective as soil larvicides against *H. collusor* in the laboratory (Mulla 1960b). However, a material to be used as a large-scale soil larvicide against eye gnats should have long-lasting effect, should not be phytotoxic at dosages required to yield gnat control, and should have no side effects.

The present work is a laboratory study and a continuation of previous work (Mulla 1960c) to determine the residual activity of some organophosphorus insecticides in soil as tested against the eye gnat, *H. cullosor*. In this way the most promising materials are quickly singled out for field evaluation. Without this procedure, evaluation of eye gnat larvicides in the field would be an expensive and, at times, impossible undertaking.

METHODS AND MATERIALS.—Three ½-gallon quantities (5.75 pounds) of Coachella fine sand containing 0.15% organic matter, dry-weight basis, were each treated with each dosage of an insecticide. Emulsifiable concentrate (EC) formulations of the insecticides containing 1.5 to 4 pounds of the actual toxicant per gallon were used in preparing dilute aqueous sprays. A 50% soluble concentrate of Trithion was diluted with isopropyl alcohol for final spraying. The materials were formulated by either commercial formulators or basic producers of the insecticides. Information on the ingredients of the formulations other than the active ingredients was not available.

The dilute sprays were applied at the rate of 100 gallons per acre or 1.16 ml. per 18-square-inch area of each soil lot. Two hours after spraying, the treated soil lots were spoon-mixed and placed in ½-gallon Mason jars. Initially, 400 ml. of water (15% moisture, dry-weight basis) was added to each treated soil lot; subsequently, 200 ml. of water was added at 2-month intervals to each lot of the remaining treated soil. The soil moisture was thus maintained at approximately 10% to 12% dry-weight basis during most of the storage period. The jars containing the moist treated soil were covered with muslin and placed in a glasshouse maintained at 88° ± 2° F. Direct sunlight was excluded from the jars by whitewashing the outside of the glasshouse.

Each material was applied at three dosages. Most of the materials were applied in the range of 1 to 9 pounds of actual material per 6-inch acre. Some of the materials such as Bayer 29493, Bayer 22408, dimethoate, American Cyanamid Compound E.N. 18133, and parathion were applied at higher dosages.

Most of the materials were evaluated for their longevity after 2, 4, and 8 months of storage; however, owing to lack of space and time, some materials were evaluated at other intervals.

For evaluating the longevity of each material against the eye gnat, one lot of the treated soil with each dosage was air-dried at room temperature not exceeding 90° F. Larval nutrients consisting of 28 grams alfalfa-leaf meal, 14 grams brewer's yeast and 14 grams sifted whole-wheat flour were added to each lot of the soil (Mulla 1960a). They were thoroughly mixed with the soil which was subsequently divided among five regular-mouth pint jars; there were thus five replications of the bioassay determinations. The treated soil—medium mixture in each of the pint jars—was moistened with 140 ml. of water. Within 3 days after the addition of water, two hundred 1- to 3-day-old eye-gnat eggs (usually stored in refrigerator) were added to each jar. Control treatments were run along with each test. The treatments were arranged in a randomized block design on a bench in a glasshouse maintained at 88° ± 2° F.

The eye-gnat colony, the eggs of which were used in these tests, was established in the fall of 1957 from wild populations collected in the Indio area of the Coachella Valley. The adult gnat emergence from the treated soil was recorded every other day. The reductions in gnat emergence from the various treatments with reference to the control treatments were indications of the presence of certain amounts of the toxicants. Dosage mortality lines of the material established against the gnat (Mulla 1960b) were utilized for calculating the amount of toxicant corresponding to a known reduction in gnat emergence. The percentage of loss was calculated from the amount of insecticide corresponding to certain reduction in gnat emergence and the original amount of insecticide applied to the soil.

When the gnat reduction is more than 98% to 99%, the amount of insecticide present in the soil cannot be determined. Appreciable loss of the material can occur, but owing to the excessive dosages used over the 100% reduction level it is not possible to ascertain such a loss. Similarly, a reduction in gnat emergence lower than 2% to 5% cannot determine the actual amount of the toxicant remaining in the soil after storage. In such cases, for practical considerations, the material is assumed to have lost its activity completely.

An indicated 100% loss of a material does not necessarily mean that the whole initial amount is lost although this may be true in certain of the tests. Because of the high biological activity of most of the materials studied here (table 1), a small portion of the initial dosages (particularly the intermediate and high ones) is necessary to obtain 90% or more reduction in gnat emergence. Therefore, a reduction in gnat emergence of 5% or lower is indicative of rapid loss of activity of the materials tested at each interval of storage.

RESULTS AND DISCUSSION.—The residual activity against insect pests of an insecticide in soil will depend

Table 1.—Initial toxicity of some organophosphorus insecticides as soil treatments in the laboratory against *H. collusor*.

INSECTICIDE	POUNDS OF TOXICANT/6-INCH ACRE	
	LD_{50}	LD_{90}
Co-Ral	0.030	0.052
Diazinon	.030	.070
E.N. 18133	.041	.092
Bayer 25141	.025	.096
Bayer 29493	.060	.131
GC-4072	.118	.188
Trithion	.136	.250
GC-3582	.184	.310
Ronnel	.178	.368
Ethyl Guthion	.110	.370
Phorate	.240	.485
Bayer 22408	.400	.740
GC-3583	.445	.805
Methyl Trithion	.400	.840
Ethion	.380	1.260
Guthion	.60	1.30
Di-Syston	.74	1.60
Parathion	3.35	4.80
Dimethoate	1.00	5.00

on many factors, some of which are the biological activity and stability of the material and its transformation due to chemical and physical processes or metabolism by micro-organisms in soil to more active or less active products. The biological and ecological patterns of the life history of a pest may also determine the degree of long-term control obtained with certain materials. Long-lasting control of soil insect pests having long life cycles, low population density, low reproductive potential and poor capability to reinfest controlled areas may not be an indication of the residual activity of an insecticide employed.

Hippelates collusor, the most common species of eye gnats in the Southwest, does not have any of these biological attributes. The duration of its life stages in soil is short (10 to 20 days); it maintains high population density in the infested areas; it has a high reproductive potential; the females are mobile and capable of infesting other areas in a short time (Mulla & March 1959). Therefore, long-lasting control of *H. collusor*, generation after generation, can be obtained only by employing effective long-lasting insecticides against this pest of public health importance.

Most of the organophosphorus insecticides evaluated as soil treatments in the laboratory indicated an exceptionally high degree of initial biological activity against the eye gnat (Mulla 1960b). However, a potential eye-gnat larvicide should have long residual activity once it is applied to the soil. Application cost of eye-gnat larvicides is usually higher than the cost of the larvicide itself and thus the inevitable emphasis on the development of residual larvicides.

The residual activity of Trithion®, Diazinon®, Bayer 25141, Co-Ral,®, and ethion was high. After 2 months of storage these materials yielded almost complete kill of the gnats at all dosage levels (table 2). After 4 months of storage Trithion, Diazinon, and Bayer 25141 produced almost complete kill of the gnats at all dosage levels. Co-Ral and ethion, however, did not produce complete kill at the lowest dosages. Complete kill was obtained, however, at the higher two dosages at this interval. Although 98% loss of Co-Ral at the intermediate dosage level was indicated at the 8-month interval, the mortality of gnats due to the remaining 2% Co-Ral was 99%. This is due to the high effectiveness of the material. The highest dosage levels of Trithion, Diazinon, Bayer 25141, and Co-Ral produced complete mortality after the 8-month interval of storage.

The residual activity of Bayer 25141 is probably due to its low rate of transformation to other compounds that break down rapidly. The transformation rate of this compound to its PS-sulfide, PS-sulfone, and thioethyl PO-sulfoxide and sulfone on cotton leaves and *in vitro* was very low (Benjamini *et al.* 1959a, 1959b). It is possible that this same mechanism of transformation and breakdown to other compounds holds true in soil.

After 2 months of storage Methyl Trithion,® Bayer 29493, and Guthion® did not produce appreciable mortality of the gnats even at the highest dosage levels (see table 2), while Bayer 22408 produced complete kill of the gnats at the highest dosage level at this interval. The first three materials have little persistence in the soil while the last-mentioned compound has a fair amount of persistence in the soil. Almost complete loss of activity was demonstrated at all dosage levels for Methyl Trithion, Bayer 29493, and Guthion at the 2-month interval of evaluation.

Phorate, Di-Syston®, ronnel, General Chemicals Compounds GC-4072, GC-3583 and GC-3582 were tested at equal rates. Among these materials GC-4072 had the longest residual activity and phorate, GC-3583 and GC-3582 were found to persist longer than Di-Syston and ronnel (table 2). However, after 2 months of storage the degree of mortality obtained with the highest dosage level of phorate and GC-3582 was below the 90% level. The over-all percentage of loss of phorate at the 2- and 4-month intervals was lower than the percentage of loss of Di-Syston, ronnel and GC-3582. It is interesting to note that GC-4072 and GC-3583 are structural isomers. The former compound has two chlorine atoms on ortho and para positions while the latter compound has the chlorine atoms on ortho and meta positions. GC-3582 is a dichlorovinyl phosphate analogue of GC-3583 and the two are closely related chemically. In terms of electron phenomena the unusual stability of GC-4072 is to be expected (Fukuto 1957).

Dimethoate, Compound E.N. 18133 and parathion were also tested at equal rates. Of these three materials Compound E.N. 18133 proved to be more persistent than the other two materials (table 3). Complete mortality of the gnats was obtained at all three dosages of E.N. 18133 at the 2-month interval of testing. At the 5-month interval of testing, only the highest dosage of this material resulted in appreciable mortality of the gnats. More than 99% loss of this material was indicated at this interval at all dosage levels. The loss of dimethoate at the 2-month interval ranged from 90% to 98% while that of parathion ranged from 38% to 100%. Although the magnitudes of losses of dimethoate and parathion were relatively low at the highest dosage levels, the materials nevertheless resulted in lower reductions of the gnats because of the lower biological activity of these two

Table 2.—Longevity of organophosphorus insecticides in soil as determined by testing against *H. collusor*.

Insecticides and Formulation	Toxicant/A. (lb.)	Average Number of Gnats/Jar (A) and Reduction (%) After Months Shown						Loss (%) After Months Shown		
		2		4		6–8				
		A.	%	A.	%	A.	%	2	4	6–8
				Experiment A						
Methyl Trithion EC 4	2	63	0	149	0	137	0	100	100	100
	4	69	0	134	2	121	8	100	100	94
	8	53	9	124	9	124	5	97	97	98
Trithion Solution 50%	2	0	100	2	99	117	11	—	—	96
	4	0	100	0	100	43	67	—	—	96
	8	0	100	0	100	0	100	—	—	—
Diazinon EC 2	2	0	100	0	100	49	63	—	—	98
	4	0	100	0	100	9	93	—	—	91
	8	0	100	0	100	0	100	—	—	—
Control	—	58	—	136	—	131	—	—	—	—
				Experiment B						
Bayer 25141 EC 2	1	0	100	0	100	50	12	—	—	99
	2	0	100	0	100	0	100	—	—	—
	8	0	100	0	100	0	100	—	—	—
Bayer 29493 EC 4	2	83	8	101	0	63	0	99	100	100
	6	101	0	100	0	58	0	100	100	100
	12	52	43	98	0	56	0	99.6	100	100
Bayer 22408 EC 2	2	91	0	120	0	59	0	100	100	100
	6	23	75	65	33	50	12	91.4	94.5	96
	12	0	100	20	79	51	11	—	95	98
Control	—	90	—	97	—	57	—	—	—	—
				Experiment C						
Co-Ral EC 1.5	1.5	0	100	36	56	84	16	—	98	99
	4.5	0	100	0	100	1	99	—	—	98
	9.0	0	100	0	100	0	100	—	—	—
Guthion EC 1.5	1.5	99	8	73	10	—	—	83	90	—
	4.5	109	0	68	16	—	—	100	92	—
	9.0	105	2	78	4	—	—	88	97	—
Ethion EC 4	2.0	2	98	21	74	67	33	—	65	87
	4.0	0	100	2	97	27	73	—	—	83
	6.0	0	100	0	100	2	98	—	—	57
Control	—	107	—	81	—	100	—	—	—	—
				Experiment D						
Phorate EC 4	2	58	48	85	15	56	0	89	94	100
	4	64	43	54	46	48	4	95	94	100
	8	12	89	36	64	67	0	94	96	100
Di-Syston EC 2	2	122	0	118	0	78	0	100	100	100
	4	124	0	103	0	70	0	100	100	100
	8	62	44	60	40	45	9	93	94	97
Ronnel EC 2	2	100	10	109	0	50	0	96	100	100
	4	105	5	92	18	60	0	98	95	100
	8	98	12	83	17	51	0	99	99	100
Control	—	111	—	100	—	50	—	—	—	—
				Experiment E						
GC-4072	2	0	100	0	100	6	95	—	—	89
	4	0	100	0	100	0	100	—	—	—
	8	0	100	0	100	0	100	—	—	—
GC-3583	2	91	25	57	20	117	0	86	85	100
	4	39	68	71	1	102	5	86	100	95
	8	1	99	68	6	109	0	85	98	100
GC-3582	2	80	34	68	6	116	0	92	95	100
	4	74	39	74	0	118	0	96	100	100
	8	19	84	19	74	109	0	96	97	100
Control	—	121	—	72	—	107	—	—	—	—

Table 3.—Longevity of insecticides in soil as determined by testing against *H. collusor*.

Insecticide and Formulation	Toxicant/A. (lb.)	Average Number of Gnats/Jar (A) and Reduction (%) After Months Shown						Loss (%) After Months Shown		
		2-3		5		8				
		A	%	A	%	A	%	2-3	5	8
				Experiment A						
Dimethoate EC 4	4	93	20	116	7	91	0	90	95	100
	8	113	3	120	4	96	0	98	98	100
	16	60	49	128	0	91	0	94	100	100
E.N. 18133 EC 4	4	1	99	108	14	84	0	—	99.5	100
	8	0	100	113	10	93	0	—	99.8	100
	16	0	100	27	79	83	1	—	99.6	100
Parathion EC 4	4	98	16	129	0	89	0	38	100	100
	8	116	0	120	4	79	6	100	92	100
	16	66	43	133	0	82	3	80	100	100
Control	—	116	—	125	—	84	—	—	—	—
				Experiment B						
Ethyl Guthion EC 2	1	2	98	22	79	21	69	—	78	82
	4	0	100	1	99	0	100	—	—	—
	8	0	100	0	100	0	100	—	—	—
Bayer 22684 EC 2	1	90	2	95	10	87	0	100	100	100
	4	93	0	99	7	85	0	100	100	100
	8	79	14	96	19	90	0	100	100	100
Control	—	92	—	106	—	68	—	—	—	—

materials against the gnats as compared with some of the other organophosphorus compounds (see table 1). Dimethoate and parathion have little persistence in the soil while E.N. 18133 has a fair amount of persistence when tested against the gnats.

Ethyl Guthion®, and Bayer 22684 were studied at equal rates. After 2 months of storage complete loss of activity was ascertained for Bayer 22684 (table 3).

Ethyl Guthion proved highly effective at the two higher dosages at all testing intervals. The lowest dosage (1 lb./acre) resulted in 9% mortality at the 2-month interval, but declined in activity at the succeeding intervals. The loss of this material at the two highest dosage levels could not be ascertained because complete mortality of the gnats was produced by these dosages.

The persistence of a very few organophosphorus insecticides has been studied under controlled conditions. Field evaluation of these toxic agents as soil treatments against a variety of soil inhabiting insects may shed some light on the residual trend of these materials. However, the degree of control of an insect pest obtained may not necessarily reflect the long term activity of the toxicant used.

Trithion, Diazinon, ethion, Co-Ral and Guthion, for example, in multiple field tests against the onion maggot, *Hylemya antiqua* (Meigen), applied at various rates and in various formulations produced effective control of the pest (Howitt 1958). The degree of control was assessed usually once, at harvest time 2 to 3 months after the date of application. From these studies it is not readily possible to determine the breakdown point of these materials although the effectiveness of some of these insecticides against the onion maggot has been clearly demonstrated during the short growing season. Of the materials tested against onion maggot, Trithion in the studies reported herein and by Menn *et al.* (1960) showed persistence in soil. Diazinon, ethion and Co-Ral also showed long persistence while Guthion was not persistent.

Turner (1957) found that phorate applied as granules did not result in good control of the clover root curculio, *Sitona hispidula* (F.). Hopkins *et al.* (1959) obtained good control of the cotton aphid, *Aphis gossypii* Glover, for 15 months with 10, 20 and 30 pounds per acre side-dressing applications of phorate and Di-Syston. Furrow treatments with these two materials at the rate of 1 to 3 pounds per acre resulted in seasonal control of the aphid. This study suggests longer persistence or greater activity and higher concentrations of the materials in the plants.

The low persistence of parathion in soil has been demonstrated in a number of studies. Parathion applied to soil as sprays at the rate of 10 pounds per acre resulted in poor control of the onion maggot 8 weeks after treatment (Howitt 1958). Lange & Carlson (1956) obtained good reduction of the wireworm, *Limonius canus* LeConte, in the laboratory by applying parathion at the rate of 5 pounds per acre. In the field, however, this material was found to have low residual activity and did not protect potatoes against the pest. Similarly, 1-pound-to 2-pound-per-acre applications of parathion resulted in poor control of the Gulf wireworm, *Conoderus amplicollis* (Gyllenhal), 6 months after treatment of potato plots. Poor control of the wireworm, *Melanotus communis* (Gyll), was obtained when the worms were exposed in soil 8 days after the application of parathion at 5 to 20 pounds per acre (Kulash & Monroe 1954). However, this poor control of the worms might have been a case of low susceptibility of the worms to parathion. Parathion residues in soil when it was applied to soil at the rate of 15.7

p.p.m. per year for 5 years declined rapidly (Chisholm *et al.* 1955). The final concentration at the end of 5 years was found to be only 0.5 p.p.m. Evidence for the translocation of parathion in beans (nonedible parts) and buckwheat was presented.

From the studies reported herein it is apparent that there exists an obvious correlation between the structure of some of the compounds and their residual activity in soil. The substitution of ethyl for methyl groups in Methyl Trithion and Guthion to yield Trithion and Ethyl Guthion, greatly enhanced the residual activity of the resulting compounds. The rate of degradation of Bayer 25198, the methyl analogue of Bayer 25141, in cotton plants was found to be greater than that of Bayer 25141 (Benjamini *et al.* 1959b). It is possible that this relationship also holds here. The rate of translocation of the former material was also found to be twice as much as that of Bayer 25141. It is possible that similar degradation rates of these two compounds (methyl and ethyl analogues) hold true when they are applied to soil.

It is not implied here that the diethyl phenyl phosphates are invariably residual toxicants in soil. The reactivity of these compounds in terms of the electronegative properties of the molecule merits consideration. Other physicochemical properties, such as hydrolysis rates under acidic and alkaline conditions, may also influence the toxicity and stability of a material (Fukuto 1957, Fukuto & Metcalf 1956). Components of the soil complex, such as moisture, temperature, soil type, plant cover, and micro-organisms inhabiting the soil environment, would undoubtedly influence the persistence and effectiveness of various insecticides against soil-inhabiting insect pests.

The chemical definitions of proprietary materials mentioned in this article are as follows:

American Cyanamid Experimental Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate)
Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate)
Bayer 22684 (*O*-methyl-*O*-(2-chloroethyl)-*O*-(2-dichlorovinyl) phosphate)
Bayer 25141 (*O,O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate)
Co-Ral (*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7yl phosphorothioate)
Diazinon (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate)
Di-Syston (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)
Ethyl Guthion (*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate)
GC-3582 (*O,O*-diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate)
GC-3583 (*O,O*-diethyl-1-(2,5-dichlorophenyl)2-chlorovinyl phosphate)
GC-4072 (*O,O*-diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate)
Guthion (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl phosphorodithioate)
Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate)
SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan)
Trithion (*O,O*-diethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate)

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Methoxychlor in Milk of Dairy Cows Dusted with the Wettable Powder[1]

G. R. DeFoliart and Donald N. Willett[2]

Abstract

Methoxychlor in amounts of less than 0.1 p.p.m. was found in the milk of herds dusted for horn fly (*Haematobia irritans* (L.)) control with approximately 10 g. per cow of 50% methoxychlor wettable powder. The contamination was presumably of extraneous origin rather than by excretion in the milk. Under practical conditions, herds are dusted at approximately 3-week intervals so extraneous contamination would be expected to occur simultaneously on only a few farms in an area. Upon dilution with other milk from the area, the slight contamination occurring on individual farms at any given time should result in methoxychlor residues of less than 0.01 p.p.m. in commercial milk.

Direct "dusting" with the dry wettable powder at 3-week intervals is the only method of applying methoxychlor to dairy cows that is registered by the U. S. Department of Agriculture and approved by the Food and Drug Administration. Methoxychlor is excreted in milk following topical application of 0.5% aqueous suspensions or emulsions (Claborn & Wells 1952, Claborn *et al.* 1960, Cluett *et al.* 1960), but Cluett *et al.* (1960) have shown that methoxychlor is not excreted in milk following topical application of the 50% wettable powder in dry condition. This method was first recommended for horn fly (*Haematobia irritans* (L.)) control by Lindquist & Hoffman (1954) and consists of sprinkling the equivalent of a heaping tablespoonful of the dry powder over the top of the animal (poll, neck, back and upper sides) and rubbing it lightly into the hair.

The tests of Cluett *et al.* (1960) were designed to determine whether methoxychlor is actually excreted in the milk and precautions were taken to avoid outside contamination. The cows were stanchioned in alternate stalls, long hair was clipped from their udders and flanks, and the applications of methoxychlor dust were made in a closed pen remote from the dairy barn. The cow's udders were thoroughly sponged and rinsed before each milking.

Few of these precautions are taken by the average dairyman under practical farm conditions, and outside contamination of milk by the dusting method is a possibility that must be considered. In tests in the fall of 1959, methoxychlor was detected in the milk of 4 of 6 cows that were dusted. Five individual samples (of 17 from the 6 cows) contained methoxychlor at rates ranging from 0.028 to 0.09 p.p.m. 20 to 44 hours following treatment. The occurrences of the contaminated samples within the 20- to 44-hour period were erratic and seemed to indicate exogenous contamination rather than excretion. The conditions of the tests were unsatisfactory, but pointed to the desirability of additional tests to determine the possibility of contamination from routes other than excretion. Accordingly, the tests described below were conducted during the summer of 1960. They were intended to determine whether the dust treatment results in contamination of milk under practical conditions of usage on the farm.

Procedure.—The tests were conducted at three dairy farms near Madison, Wisconsin. On these farms, the milk is accumulated in bulk tanks and hauled away in a tank truck on alternate days. The dusting and milk sampling operations were made to conform with the regular milk-hauling schedule on each farm so that the first posttreatment milk was introduced into an empty tank. In other words, the pretreatment milk sample used as a blank was drawn from the bulk tank and the herd dusted during the morning of a day when the tank was scheduled to be emptied. The milk in the tank, after introduction of the milk from the evening milking, then represented only the milk from 0 to approximately 12 hours following dusting. The pretreatment samples from the various farms were designated B1, O1, and Z1 from Farms B, O, and Z, respectively. The 0- to 12-hour posttreatment samples were accordingly designated B2, O2, and Z2; the 0- to 24-hour posttreatment samples (a composite of two milkings) were designated B3, O3, and Z3; and the 0- to 48-hour posttreatment samples (a composite of four milkings) were designated B4, O4, and Z4.

The dust was applied to the cows' backs and rubbed in lightly as they stood in the stanchions following the morning milking. Details of the dust formulation and amount applied per cow are given below with information on the individual farms.

Every effort was made to insure against possible contamination of the collecting bottles. The individual doing the dusting drove a vehicle carrying the insecticide while the person doing the milk sampling drove an uncontaminated vehicle. The two did not associate closely during the period, one working in the barn, the other in the milk house. The milk was collected in 4-ounce plastic bottles by dipping them well under the surface of the milk while the tank agitator was in operation. The samples were then returned to Madison and refrigerated. All samples were taken as duplicates for later analysis by the Feed and Fertilizer Laboratory of the Wisconsin Department of Agriculture and by the DuPont Company.

The work was done on the following farms: Farm B: 39 Holsteins were dusted at the rate of 27.3 g. of dust per cow. This is a heavy overdose, as only 10 g. per cow is recommended. However, it is an amount that could be interpreted as a "heaping tablespoonful." Commercial 50% methoxychlor wettable powder was used. The herd was treated at 7:30 a.m. on August 4. It had been dusted with methoxychlor twice previously during the summer but not within 3 weeks prior to the beginning of this test; Farm O: 29 Holsteins were dusted at 9.7 g. per cow. Commercial methoxychlor was used. The application was made at about 8:00 a.m. on August 4. Methoxychlor had not been used previously during 1960; Farm Z: 32 Holsteins were dusted at 11.4 g. per cow. A methoxychlor formulation in which the wetting agent had been deleted was used. The application was made at 8:00 a.m. on August 5. Methoxychlor had not been used previously during 1960.

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[2] Associate Professor, Department of Entomology, University of Wisconsin, and Head, Feed and Fertilizer Section, Wisconsin State Department of Agriculture, respectively.

Table 1.—Methoxychlor found in milk from three herds dusted for horn fly control.

Methoxychlor (50%) W.P./Cow (grams)	Sample No.	Wisconsin μg. (net)/100 g. of milk[a]	Wisconsin p.p.m.	DuPont p.p.m.[b]
27.3	B-2	9.6	0.096	0.03
	B-3	9.6	0.096	0.03
	B-4	9.1	0.091	0.07
9.7	O-2	4.9	0.049	0.03
	O-3	3.9	0.039	0.02
	O-4	4.4	0.044	0.03
11.4[c]	Z-2	7.0	0.07	0.03
	Z-3	6.0	0.06	0.03
	Z-4	8.0	0.08	0.03

[a] Corrected for amounts shown in text for pretreatment samples, B-1, O-1, or Z-1, respectively.
[b] Corrected for 0.02 p.p.m. blank in pre-treatment milk.
[c] Without wetting agent.

The colorimetric method of Cluett *et al.* (1960) was followed except for a few minor changes. To prevent possible loss of methoxychlor during the evaporation of the nitromethane, the samples were placed in Erlenmeyer flasks and evaporated in a water bath at temperatures not exceeding 70° C. As the extract approached dryness it was removed from heat and carefully evaporated at room temperature with the aid of a gentle current of air. To form the colored complex 20 ml. of a sulfuric acid color reagent (above 90% sulfuric acid containing 10 mg. of ferric chloride per liter) was added to the residue and the mixture allowed to stand 1.5 hours with occasional stirring.

A calibration curve was prepared using standard dehydrohalogenated methoxychlor. From this curve the apparent methoxychlor present was determined.

The apparent methoxychlor (p.p.m.) in the milk samples drawn before treatment and used as blanks was as follows:

University of Wisconsin	0.007
Sample B-1	.022
Sample O-1	.028
Sample Z-1	.017

These blanks were used to correct the methoxychlor values found in the respective herds. Milk from the untreated herd of the University was used for recovery studies. Recovery averaged 103.7% as follows:

Methoxychlor (μg.) Added	Found	Recovery (%) (Blank Deducted)
3	3.3	110
7	7.5	107
12	11.3	94

DISCUSSION OF RESULTS.—The results of analyses of the milk samples by both the DuPont Company and the Feed and Fertilizer Laboratory of the Wisconsin Department of Agriculture show small amounts of methoxychlor in all posttreatment samples (table 1). The differences in the amounts found by the two laboratories are not readily explained, but would appear of not very great importance, as they are near the lower sensitivity levels of the method. It is surprising, however, to find that the 27.3 g. dosage per cow used on Herd B resulted in only slightly greater contamination than the dosage of 9.7 and 11.4 g. per cow. The 2½ to 3 times greater volume of dust used on Herd B would seem to offer a greater chance of general contamination from airborne particles and particles adhering to lower parts of the animals as well as possibly resulting in excretion in the milk at amounts near the detectable level. Cluett *et al.* (1960) found that increasing the amount of dust applied from 10 g. to 40 g. per cow resulted in the excretion of detectable amounts of methoxychlor in the milk (up to 0.05 p.p.m. on the third day following treatment) at the higher dosage. They did not test intermediate rates of application, however, so the minimum rate that may produce a detectable level through excretion is not known.

The similarity of contamination levels from the applications of 27 g. and approximately 10 g. indicates that the levels shown in table 1 will be the expected result of the dusting method within the range of application rates tested, unless extra care is exercised in applying the dust at the currently recommended rate of 10 g. per cow.

Surveys by the Food and Drug Administration in 1955 and 1958 (Clifford 1957, Clifford *et al.* 1959) did not reveal any important contamination of raw market milk by methoxychlor although it has been used on dairy cattle as a dry powder during these years. Failure to find methoxychlor in the 1958 survey, conducted from August through November, indicates that little if any detectable contamination of market milk is occurring even though contamination with minute amounts of methoxychlor may occur at the individual farm level. Considering the infrequency of dust application (approximately 3-week intervals), the prospect is unlikely that a large percentage of dairymen in an area will dust their herds within any one span of 2 or 3 days. Consequently, dilution in the tank truck should ordinarily result in methoxychlor residues of less than 0.01 p.p.m.

Wisconsin recently registered methoxychlor for use as a dust treatment on dairy cows. This was done on the basis that the total contamination at the farm level indicated by these tests should not be expected to result in market milk residues considered significant by current standards. Nevertheless, further tests on dust application methods and amounts of dust applied would seem desirable. Limited observations following dusting of herds by farmers indicated horn fly control can be obtained with less than the recommended 10 g. per cow, but the tests were not carefully controlled and the exact amounts used were not determined. Also, it seems doubtful, considering the habits of horn flies, that it is necessary to apply the dust over such a large area of the animal. It seems likely that horn flies will contact 5 g. of dust applied only to the upper shoulder and the back anterior to the loin nearly as readily as 10 g. over a larger area.

An inference may be drawn from these tests that in the future more attention should be given to the possibilities of exogenous contamination with insecticides contemplated for use in dairy barns and on dairy cows. Methoxychlor dust at the recommended level does not result in excretion at detectable levels, yet these tests show that contamination may occur anyway. The same situation may well exist with other insecticides, particularly those used in cattle fly sprays, that have received

registration for use in dairies and on dairy cows on the basis of data which measure excretion only.

The procedure followed in securing federal registration for Lethane 384 (Gordon *et al.* 1960), the most recently registered material for use as a spray directly on dairy cattle, is an example. The cows were sprayed in a building well separated from the milking shed and the individual who did the spraying had no further responsibilities in the test. During spraying the udders were completely enveloped in two plastic bags, one inside the other. Following the application and settling of the spray, the outer plastic bag was removed and after the animal had been led to the milking shed, the inner plastic bag was removed. During spraying a canvas bag was kept over the nose and mouth of each animal. The duties following spraying were performed by another individual, but he did not milk the animals or handle any of the collection apparatus. After he had placed a surcingle strap over the back to hold the milking apparatus, a third individual took over, washed the teats with sanitizing solution, and attached the milker without coming into personal contact with the animal. These are typical of precautions taken to avoid extraneous contamination. Although such a procedure shows whether an insecticide may be excreted in the milk it gives only slight indication as to whether contamination may occur under practical conditions.

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The Persistence of Gypsy Moth Parasites in Heavily Sprayed Areas on Cape Cod, Massachusetts[1]

Philip B. Dowden[2]

Abstract

Intensive spray programs have kept the gypsy moth (*Porthetria dispar* (L.)) at extremely low levels on Cape Cod since 1948. In spite of this most of the parasites introduced against this pest were recovered there in 1960.

Ever since 1944, when DDT was first dispersed by airplane for control of forest insects (Craighead & Brown 1945), there has been concern about the effect that such a powerful insecticide might have on woodland fauna in general, including natural control agents such as beneficial insect parasites and predators. Detailed observations that year, and subsequently, have led to the conclusion that no serious changes in the prevailing insect fauna have followed the occasional aerial application of 0.5 to 1 pound of DDT per acre of woodland in approximately 1 gallon of fuel oil or a not more injurious solvent.

Perhaps the principal reason for this conclusion is that few, if any, species are known to have been actually eliminated over wide areas in this manner; and even species that have been tremendously reduced in number have generally returned rapidly to normal endemic numbers.

In forest insect control programs, species against which spraying has been directed have usually been reduced to extremely low population levels. In most cases populations of their principal parasites and predators have probably been reduced accordingly through direct mortality or loss of the hosts upon which they depended for food and propagation. The latter has undoubtedly been the most important because most beneficial species are present in several developmental stages when a spray is applied and those within a cocoon or within a host which survives are protected from the insecticide.

Generally speaking, populations of beneficial species are probably reduced very little more by spraying operations than they are by the natural collapse of a heavy infestation. An interesting observation is reported by Macdonald (1959), who noted that percentage parasitism of the spruce budworm by *Apanteles fumiferanae* Roh. has consistently increased after spraying operations against that pest in New Brunswick.

Most observations of parasite and predator populations in sprayed woodlands have been made in relatively small or broken-up areas where a fairly rapid return to normal populations has been insured through an influx from unsprayed areas, or in larger woodland regions where a substantial host population has survived application of the insecticide. The objective of such spraying as this has usually been to reduce pest populations—not to eradicate. So there is still considerable speculation as to how parasite and predator populations might fare in large areas where intensive spray programs aimed at eradication are conducted.

An opportunity to make limited observations on such an area was presented in the summer of 1960. Several

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[2] Formerly on the staff of the Forest Insect Laboratory of the Northeastern Forest Experiment Station, U. S. Forest Service, New Haven, Connecticut; transferred in January 1961 to the Entomology Research Division of the Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

small infestations of the gypsy moth (*Porthetria dispar* (L.)) were located on Cape Cod through an aerial survey conducted by the Plant Pest Control Division of the Agricultural Research Service, and collections were made in three of these infested areas to recover parasites.

AERIAL SPRAY PROGRAM ON CAPE COD AND VICINITY.—Cape Cod is a large, well-defined, rather isolated area comprising 255,360 land acres, of which 200,000 acres are wooded. During past years it has suffered so severely from gypsy moth defoliation that in 1949 an attempt was made to eradicate the pest on the whole Cape by spraying all of the woodland by airplane, using ½ to ¾ pound of DDT in 1 gallon of fuel oil per acre. Complete spray coverage was very nearly accomplished, and the small area that was not sprayed that year was treated in 1950. Since that time annual surveys have been conducted by local, state, and federal employees to locate any incipient outbreaks. Those found have been sprayed the following season, soon after egg hatch in the spring.

The principal methods relied upon for the annual surveys have been aerial observation and trapping of male moths. From the air, defoliated areas can be spotted readily. A ground crew then determines whether the damage was caused by the gypsy moth; if so, the area involved is marked for treatment. The trapping method has been used extensively by the Plant Pest Control Division to locate very light infestations where no defoliation occurs. Traps are baited with female sex attractant. Since the capture of a male moth is a clear indication that the pest completed its development in the vicinity of the trap that season, an effort is made to locate the actual feeding area. This has not always been possible, but in any event the standard procedure has been to spray about 160 acres around traps where males were caught.

The program of spraying on Cape Cod was supplemented substantially by similar work in Plymouth County, which lies directly to the west of the Cape. The whole of Plymouth County was sprayed in 1950; and all areas of the County adjacent to the Cape, where moths were trapped in 1952, were resprayed in 1953. Nantucket Island was sprayed in 1951 and 8,545 acres on the west side of Martha's Vineyard were sprayed in 1956. Only a few miles of open water separate these bodies of land from the Cape.

This work was further supplemented in 1955 when the wooded area on the Cape was sprayed to control the pine looper, *Lambdina athasaria pellucidaria* (G. & R.). In this program, about 100,000 pounds of DDT in 131,000 gallons of fuel oil were sprayed on 203,000 acres between July 18 and August 6. This was too late to control the gypsy moth effectively, but it was probably early enough to kill a substantial proportion of late-developing larvae. On July 12 in 1960, for instance, about 50% of the population was in the larval stage and 50% in the pupal stage.

C. S. Hood, Department of Natural Resources, Division of Forests and Parks, Commonwealth of Massachusetts, kindly supplied data showing the extent of these spray operations. The whole program is summarized from these data in table 1.

COLLECTION AREAS.—In spite of this intensive spray program conducted on Cape Cod and adjacent areas from 1948 through 1960, three small but heavy woodland infestations were located there in 1960. Two were in Barnstable, not more than a mile apart, and approximately a mile from areas sprayed in 1959 and 1960. One of these areas was designated as Barnstable No. 1, the other as Barnstable No. 2. The third area was in Truro, about 40 miles east of Barnstable. It also was within a mile or so of areas sprayed in 1959 and 1960.

The three infestations were similar. Each was located on a low hill. In each case, 3 or 4 acres were heavily infested, while the surrounding growth appeared to be entirely uninfested. Judging from the remains of old egg clusters, Barnstable No. 1 may have been infested 2 years, Barnstable No. 2 for 3 years, and Truro only 1 year (1960). These observations lead one to suspect that these infestations developed from wind dispersion of first-instar larvae from the older infestations early in the spring before they were treated.

RECOVERY OF PARASITES.—Collections of 600 larvae and 600 pupae were made at each of the infestations in Barnstable on July 11, and a similar collection was made at Truro on July 12. They were taken to the Northeastern Forest Experiment Station's Forest Insect Laboratory in New Haven, Connecticut, for rearing and the recovery of parasites. Since most of the larvae were full grown, they required very little further attention. On September 25 the three areas were inspected again, and approximately 2,000 eggs were collected in each for the recovery of egg parasites. Recoveries from all collections are summarized in table 2.

The parasite recoveries listed in table 2 show that all

Table 1.—Aerial spray program conducted against the gypsy moth on Cape Cod and adjacent areas, 1948–1960.

AREA	YEAR	ACREAGE SPRAYED	DDT APPLIED (LB.)	REMARKS
Cape Cod	1948	7,864	3,932	Two large blocks, Sandwich and Brewster areas.
	1949	229,831	114,915	All of woodland on Cape except 7,000 acres in Truro and Wellfleet.
	1950	10,541	10,453	Completion of 1949 program plus 7 small areas resprayed.
	1953	5,760	2,880	36 areas of 160 acres each.
	1955	203,074	98,262	Sprayed for pine looper.
	1956	7,760	3,900	21 areas, mostly 300 acres each.
	1958	1,600	1,630	2 applications on one block in Brewster-Orleans area.
	1959	600	450	1 area in Truro, 1 in Barnstable.
	1960	1,100	850	Approximately same areas as in 1959.
Nantucket Island	1951	31,071	31,071	Complete coverage.
Martha's Vineyard	1956	17,000	8,545	West side of island.
Plymouth County	1950	424,469	286,000	Virtually complete coverage+2,000 acres.
	1953	7,970	3,985	48 areas, mostly 160 acres each.
	1956	69,029	34,515	112 areas, mostly 300 acres each.

Table 2.—Recoveries from 600 larvae, 600 pupae, and 2,000 gypsy moth eggs collected at each of 3 areas on Cape Cod in 1960.

RECOVERIES OF MOTHS AND PARASITES	BARNSTABLE No. 1	BARNSTABLE No. 2	TRURO
Moths	881	950	906
Parasites:			
Ooencyrtus kuwanai (Howard)	common	common	common
Apanteles melanoscelus (Ratzeburg)	16	18	2
Compsilura concinnata (Meigen)	58	41	15
Exorista larvarum (L.)	1	0	3
Parasetigena silvestris R. D.	2	6	7
Sturmia scutellata R. D.	60	17	93
Borrelina reprimens Holmes	0	19	0
Theronia atalantae (Poda)	0	1	0

except two of the important species introduced from abroad and successfully established in this country against the gypsy moth have been able to maintain themselves on Cape Cod in spite of the intensive aerial spray program conducted there during the past 12 years. The two species conspicuous by their absence are the egg parasite *Anastatus disparis* Ruschka and the predaceous beetle *Calosoma sycophanta* (L.) They will be discussed briefly later. Figures in table 2 cannot be used to estimate percentage parasitism because the collections were made too late in the season to obtain a maximum recovery of several species.

INDIVIDUAL SPECIES.—Adult *Ooencyrtus kuwanai* were commonly observed ovipositing in gypsy moth eggs in all three areas in September. No figures for percentage parasitism were obtained in 1960, but parasitism of about 15% was recorded at Truro in 1956, and there is no doubt that this species is still an important parasite on the Cape. It is particularly effective in small egg clusters.

Apanteles melanoscelus is a parasite of small gypsy moth larvae. It emerges from the host and overwinters as a cocoon. At the time the collections were made, cocoons had already been formed. Therefore, figures in table 2 represent only the number of *Apanteles* cocoons found when collections were made.

Compsilura concinnata was well represented at each plot, but collections were made too late to get accurate figures of percentage parasitism by this species.

The recoveries of *Exorista larvarum* and *Parasetigena silvestris* Robineau-Desvoidey are the first recoveries of these species on Cape Cod. In 1941 a special survey showed that both were widely distributed in New England, but neither one was reared from collections made at four different points on the Cape.

Sturmia scutellata Robineau-Desvoidey maggots do not emerge until gypsy moth pupae are formed, so the records for this species are accurate. *Sturmia* never was very plentiful in southeastern Massachusetts. These figures compare favorably with others available regarding parasitism by *Sturmia* in that region.

Borrelina reprimens is the pathogen that causes the polyhedrosis virus disease of the gypsy moth. It was present in collections from only one area, which is believed to have been infested 2 or 3 years longer than the others.

Theronia atalantae is a common native parasite of Lepidopterous pupae.

Anastatus disparis and *Calosoma sycophanta* were not recovered. Both were plentiful on the Cape in the past, but both are apt to be rare in light, incipient infestations. The limited observations made this one season certainly do not prove that they are not present on the Cape now, but populations of both species probably were reduced very severely by the spray programs. *Anastatus* disperses very slowly, and this probably has limited its chances of survival. *Calosoma* apparently requires heavy infestations to build up high populations. Nevertheless it would seem that its feeding habits were diverse enough for it to maintain itself under adverse conditions.

DISCUSSION.—It is interesting to speculate as to how the various gypsy moth parasites have been able to survive on Cape Cod. Strongly polyphagous species such as *Compsilura concinnata* and *Exorista larvarum* have probably had little difficulty finding suitable hosts, regardless of the spray program. That is hardly true, though, for *Ooencyrtus*, *Parasetigena*, and *Sturmia*. Though none of these species are considered strictly monophagous, they have never been reared from field collections of any host except the gypsy moth in this country, in spite of the fact that a great deal of effort has been expended in an attempt to recover them from other native species. *Apanteles melanoscelus* has been reared occasionally from field-collected *Hemerocampa leucostigma* S. & A. *Ooencyrtus* has been reared from several hosts under laboratory conditions. So far as is known, the virus *Borrelina reprimens* attacks only *Porthetria dispar*.

If *Ooencyrtus*, *Apanteles*, *Parasetigena*, and *Sturmia* actually are very nearly dependent on the gypsy moth as a host, it must be concluded either that they have unexpected ability to disperse and find their quarry or that the spray program has failed to eliminate their supply of hosts. It may be that both of these conclusions are correct. The fact that it has been necessary to respray small areas where local infestations have appeared, during the last 12 years, suggests that the gypsy moth has never been eradicated from Cape Cod. On the other hand anyone who has participated in the surveys and has seriously hunted for the gypsy moth on the Cape must marvel at the ability of these parasites to find the small, apparently isolated infestations that the intensive surveys have turned up. The wilt disease (*Borrelina*) is in another category. It is very interesting that it was present and there may be some significance in the fact that it appeared to be in only one area. It is not known how it is dispersed.

The fact that these natural control agents can survive and disperse while an intensive aerial spray program is being conducted is extremely gratifying from many standpoints, even if it seems to indicate that the gypsy moth cannot be eradicated on a large area by an intensive program of aerial spraying of DDT such as that conducted on Cape Cod during the past 12 years.

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The Gypsy Moth Egg Parasite, *Ooencyrtus kuwanai*, in Southern Connecticut in 1960[1]

PHILIP B. DOWDEN[2]

ABSTRACT

Field observation and laboratory rearing indicate that *Ooencyrtus kuwanai* completes one spring and two and a partial third summer generation as a parasite of the gypsy moth in southern Connecticut. Parasitization has averaged 30% to 40%. This seems to be about the maximum that can be attained in medium-sized egg clusters.

Hitchcock (1959) concluded that the imported gypsy moth egg parasite *Ooencyrtus kuwanai* (Howard) completed only one summer generation in southern Connecticut. Many years ago the writer was familiar with the large-scale propagation of this species at Melrose Highlands, Mass., and accepted Crossman's (1925) conclusion that *Ooencyrtus* probably completed four summer generations. These viewpoints are so divergent that it seems worth while to clarify the situation.

NUMBER OF GENERATIONS ANNUALLY.—*Ooencyrtus* is a solitary internal parasite of gypsy moth eggs. Occasionally it develops as a hyperparasite, and occasionally two individuals develop in one egg. About 20 days are required for it to complete development from egg to adult in August. Under laboratory conditions propagation may be continuous. Nine successive generations were reared in a partially heated laboratory room from November 1959 to October 1960, and four summer generations completed development outdoors in 1960. *Ooencyrtus* adults are long-lived, and there is considerable overlapping of generations. In nature the winter is spent as an adult, and a spring generation is completed on overwintering host eggs.

Laboratory rearing is only an indication of what occurs under field conditions; so additional observations were made in both field and laboratory.

Ooencyrtus adults were first seen ovipositing in fresh gypsy moth eggs in 1960 on July 22 at Oxford, Conn. These adults were presumably from the spring generation—or they may have overwintered. Gypsy moth eggs had not been available long enough for a generation to have developed earlier. A collection of 5,825 gypsy moth eggs made at that time yielded 836 *Ooencyrtus* adults (14% parasitism); 581—nearly 75%—emerged on August 1, 2, and 3. These adults oviposited readily when given fresh gypsy moth eggs, and there seems no reason to doubt that *Ooencyrtus* adults emerging in the field at that time would not have oviposited just as readily. Second-summer-generation adults began emerging on August 20.

On August 23 a collection of eggs was made at Oxford to check on emergence of *Ooencyrtus* from eggs attacked in the field, and subsequent collections were made on September 6, September 21, and October 10. Each egg cluster was isolated and the emerging parasites were removed daily. When emergence was complete, all eggs in each cluster were examined to determine total parasitization and the number of eggs that had produced *Ooencyrtus* before the collection was made. Results are summarized in table 1. Unfortunately vandals destroyed one-half of the egg clusters in the collection of October 10. Data in table 1 indicate that a high proportion of *Ooencyrtus* adults emerged before the middle of September.

Table 1.—Parasitization of gypsy moth eggs at Oxford, Conn., in 1960, and percentage of emergence at time of collection.

Date of Collection	Number of Clusters	Total Eggs	Per Cent Parasitized	Per Cent *Ooencyrtus* Emerged	
				Before Collection	After Collection
Aug. 23	12	3,689	35	33	67
Sept. 6	12	3,516	38	74	26
Sept. 21	12	3,719	41	89	11
Oct. 10	6	2,125	37	97	3

Daily emergence records used in connection with records regarding developmental period should indicate when oviposition occurred. An attempt was therefore made to determine the developmental period from August through September. Fresh eggs were exposed almost daily from August 30 to October 1 to 30 females which had emerged August 29 (lots A1–A15), and a similar test was run with second-generation females which emerged September 8 (lots B1–B6). Results are shown in table 2.

The eggs used in these experiments were laboratory-reared unparasitized eggs, which were cleaned and glued

Table 2.—Time required for emergence of adult *Ooencyrtus* from gypsy moth eggs exposed on different dates in 1960.

Lot No.	Exposure Date	No. of Parasites Emerged	Parasitization (%)	Days Required for Emergent		
				First	Last	Average
A1	Aug. 30–31	117	48	19	25	22
A2	Aug. 30–Sept. 1	246	98	20	31	23
A3	Sept. 1–2	250	99	20	30	25
A4	Sept. 2–3	281	97	23	29	28
A5	Sept. 3–5	130	100	26	31	28
A6	Sept. 5–6	245	99	25	37	27
A7	Sept. 6–8	273	96	25	37	30
A8	Sept. 8–9	165	100	29	37	31
A9	Sept. 9–11	120	96	31	39	34
A10	Sept. 12–14	132	50	33	43	35
A11	Sept. 14–15	56	31	33	57	40
A12	Sept. 15–16	18	?	42	56	46
A13	Sept. 16–19	17	11	48	67	61
A14	Sept. 19–20	7	5	64	74	70
A15	Sept. 20–21	0	—	—	—	—
B1	Sept. 9–11	116	?	31	39	36
B2	Sept. 12–14	141	?	34	53	36
B3	Sept. 14–15	162	?	33	51	38
B4	Sept. 15–16	87	?	38	58	45
B5	Sept. 16–19	147	?	44	65	52
B6	Sept. 20–21	38[a]	28	—	—	—

[a] Emerged only after being brought into laboratory.

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[2] Formerly on the staff of the Forest Insect Laboratory of the Northeastern Forest Experiment Station, U. S. Forest Service, New Haven, Connecticut; transferred in January 1961 to the Entomology Research Division of the Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

to small pieces of cardboard to facilitate oviposition. All were held outdoors. The last adult emerged December 2 from eggs attacked September 19–20. In addition, 38 adults emerged from one lot of eggs attacked September 20–21, which was brought inside on December 2.

Using data from table 2 and emergence records from field-collected eggs, it is possible to determine fairly accurately when oviposition occurred in the field. Eggs collected on September 21 from which *Ooencyrtus* had already emerged, for instance, must have been attacked before September 1, because at that time of year at least 20 days are required for development. The average is 25 days. Therefore at least 89% of the parasitized eggs collected on September 21 were parasitized before September 1. Since no *Ooencyrtus* emerged outdoors from eggs attacked in the laboratory after September 20, it is believed that the September 21 collection indicates a maximum figure for late oviposition and that actually an even higher proportion of the parasitized eggs were attacked before September 1.

With the foregoing facts in mind, it seems clear that two and a partial third summer generation of *Ooencyrtus* completed development at Oxford in 1960. Adults of the first generation were present in large numbers on August 1, second-generation adults were abundant on August 23, and their progeny could be expected to emerge from mid-September to the end of the season as indicated by collections made September 21 and October 10 and noted in table 1.

Under natural conditions there certainly would be a considerable overlapping of generations, and some of the late-emerging adults might have been progeny of late-emerging first-generation females. It seems certain, though, that many were progeny of second-generation females, because newly emerging adults consistently oviposited within 24 hours throughout the whole season regardless of when they emerged or from which generation they developed.

PERCENTAGE PARASITIZATION.—Percentage of parasitization was determined for egg clusters held for *Ooencyrtus* emergence, and for several lots examined directly. Results are summarized in table 3.

The fact that percentage of parasitization varied very little in collections made from August 23 to October 10 is rather surprising. There are four factors that seem important in this connection. (1) *Ooencyrtus* is small, and as a rule it apparently is incapable of attacking more than the outermost layers of eggs. Maximum parasitization is thus limited to a relatively small proportion of the eggs in large clusters. (2) *Ooencyrtus* was exceptionally abundant at Oxford in 1960. From August 1 until the end of the season, several *Ooencyrtus* adults could be seen on almost every egg cluster during warm, sunny weather. (3) *Ooencyrtus* avoid ovipositing in parasitized eggs (Lloyd 1938). (4) *Ooencyrtus* exhibits a tendency to concentrate on individual clusters. Out of 10 clusters examined on August 12, for instance, two were unparasitized. This tendency was also manifest in the later collections: from some clusters practically all the parasites emerged at an early date; from others at a much later date. Considering all of these factors, one may conclude that *Ooencyrtus* parasitized very nearly all of the eggs it was capable of attacking at a surprisingly early date in Oxford in spite of the fact that adults seen were actively running over egg clusters until late into November.

Table 3.—Percentage parasitization of gypsy moth eggs collected at Oxford, Connecticut, in 1960.

DATE COLLECTED	NO. OF CLUSTERS	TOTAL EGGS	PER CENT PARASITIZED
July 22	20[a]	5,825	14
August 12	10	2,954	11
August 18	[b]	1,115	28
August 23	12	3,689	35
September 6	24	6,518	40
September 21	24	7,051	40
October 10	18	5,951	37

[a] *Ooencyrtus* adults were observed on each of these clusters.
[b] Eggs drawn from a mixed lot of 10 clusters.

Table 4.—Per cent parasitization of gypsy moth eggs in clusters of different sizes at Oxford, Connecticut, 1960.

GROUP	NO. OF EGGS PER CLUSTER	PARASITIZATION	
		Per Cent	*Range*
1	98–200	48.6	39–58
	201–250	46.3	33–59
2	251–300	42.8	38–50
	301–350	42.2	30–51
3	351–400	34.3	25–49
	401–450	35.3	28–46
4	451–800	27.6	24–31

CORRELATION BETWEEN SIZE OF CLUSTER AND PER CENT PARASITIZATION.—If the assumption is correct that the percentage of eggs attacked by *Ooencyrtus* depends to a large extent upon the number of eggs that a female can reach with her ovipositor, there should be a correlation between egg-cluster size and percentage of parasitization. This was the case, and a highly significant correlation existed between these two variables ($r = .68$) for 64 egg clusters collected at Oxford between September 6 and October 10. The relationship is more apparent if the egg clusters are arranged in groups according to size. This is shown in table 4, in which four groups are readily separated from each other.

DISCUSSION.—*Ooencyrtus kuwanai* has been abundant in gypsy moth infestations in southern Connecticut during 1957–58. In fact, in the older infestations it has not been unusual to see several adult *Ooencyrtus* on practically every egg cluster on warm, sunny days in late summer. Parasitization has averaged from 30% to 40%. *Ooencyrtus*' potential seems even higher for it can complete two or three summer generations. This seems to be about the maximum parasitism that can be attained in medium-sized egg clusters, though, because the parasites are small and appear incapable of attacking more than the outermost layers of eggs. Small, thin clusters are more vulnerable and more heavily attacked than large, compact ones. It is unfortunate that some other agent does not break up the clusters and make the eggs more available. In central Europe several species of Dermestidae commonly tunnel into gypsy moth egg clusters. *Ooencyrtus* has now

been established in Europe and it will be interesting to note whether it becomes more effective there than it is in New England.

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Introduction, Colonization, and Establishment of *Bathyplectes curculionis*, a Parasite of the Alfalfa Weevil in the Eastern United States[1]

BENJAMIN PUTTLER, D. W. JONES, and L. W. COLES, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Bathyplectes curculionis (Thomson), a parasite of the alfalfa weevil (*Hypera postica* (Gyllenhal)), was introduced into the eastern United States in 1959 and 1960. Recoveries in 1960 from 1959 releases indicate its establishment in several release sites in New Jersey, and at one locality each in Delaware and Virginia. Methods of collecting, rearing, release, and recovery of the parasite are discussed.

Since its introduction into the eastern United States in 1951, the alfalfa weevil (*Hypera postica* (Gyll.)) (Poos & Bissell 1953), has become perhaps the most serious pest of first-cutting alfalfa and at times may also do considerable damage to the second cutting (app 1959). It has spread rapidly in the ensuing years and is now firmly established from Georgia to Massachusetts along the Atlantic seaboard with scattered infestations in Alabama, Kentucky, and Tennessee (U. S. Agricultural Research Service, Plant Pest Control Division 1959).

Pest species introduced into a new area, from either a foreign source or from a distinct locality within the country, are seldom accompanied by any of their natural enemies. The alfalfa weevil invasion of the eastern seaboard was no exception to this rule. The most important natural enemy of the weevil is *Bathyplectes curculionis* (Thomson). This parasitic wasp is an endoparasite of the weevil larvae that was introduced from Italy into Utah from 1911–1914 (Chamberlin 1926).

The parasite readily became established and has spread naturally or was colonized in newly infested areas so that its distribution now encompasses practically all of the infested areas in the Western United States (Clausen 1956). *B. curculionis* has been reported to be of considerable importance in the biological control of the weevil in the Great Basin area of the United States (Hamlin *et al.* 1949), and in northern California (Michelbacher 1940, 1943). In southern California *curculionis* is established on the Egyptian alfalfa weevil (*Hypera brunneipennis* (Boh.)), a species related to *H. postica* (van den Bosch 1953). However, a lesser degree of control is exerted over *H. brunneipennis* because this host exhibits a partial immunity to attack by *curculionis* (van den Bosch & Dietrick 1959).

The first attempt to introduce *B. curculionis* into the Eastern United States was initiated in 1953 and continued through 1955. Cocoons of the parasite were obtained from Utah, and a total of 5,545 adults were released at three localities in Maryland and one in Delaware. Recoveries were attempted during 1955–1957. Only six *curculionis* cocoons were found, five in 1955 and one in 1956. None were found in 1957. More than 143,500 weevil larvae were collected and reared during this period, and it was concluded that the first attempt to introduce the parasite had failed (B. A. App, personal communication). In 1957 a second attempt was made to establish *curculionis* in the eastern alfalfa weevil infested area. This report covers the results of work that started in 1958 and was continued through 1960.

SOURCE OF PARASITES.—Practically all *B. curculionis* colonized both in 1959 and 1960 were collected from alfalfa fields infested with larvae of Egyptian alfalfa weevils and reared by the senior author while he was stationed in California. Additional stock was obtained from H. L. Parker of the European Parasite Laboratory, Paris, France. It seemed advantageous to rear *curculionis* from the Egyptian alfalfa weevil in California for the following reasons: (1) Alfalfa fields with a previous history of high populations of both host and parasite were readily accessible within a relatively short traveling distance; (2) the parasite was obtainable earlier in the season than in any other area where it is distributed in the Western United States; (3) the possibility of obtaining a greater percentage of nondiapausing *curculionis* in the first generation (1959) was enhanced, for in southern California where *curculionis* usually undergoes two generations a year (van den Bosch & Dietrick 1959), the females oviposit in late winter and early spring in weevil larvae at any stage of development, and death of the host takes place after it has spun its cocoon. Most *curculionis* of this winter-spring generation do not undergo diapause but immediately emerge and attack the remaining host larvae. These then develop and form cocoons in which the insects remain in diapause until the following spring.

COLLECTION AND REARING.—In April 1958, 850 *B. curculionis* were reared from weevil larvae collected by sweeping a number of alfalfa fields throughout San Diego

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County, Calif. Since the host generation was almost complete, all reared *curculionis* went into diapause. The 1959 collections were more intensified. After a survey in San Diego County, alfalfa fields were chosen which in January contained a few adult parasites and 1 to 2 per sweep of weevil larvae. In February, as the weevil population increased (5 to 10 per sweep), weekly collections were initiated and continued until the middle of April.

Field-collected weevil larvae were brought back to the laboratory and placed in rearing cages. They were fed alfalfa daily until the majority had either produced parasite cocoons or weevil pupae. This usually took from 7 to 10 days under laboratory conditions of 72° to 76° F. and 30% to 50% relative humidity. Parasite cocoons were then collected and held for shipment. The remaining stock was held an additional 7 days to obtain adults of nondiapausing *curculionis*. These emerged adults were fed honey and placed at 40° F. until ready for shipment. As a result of the 1959 collections, 7,295 *curculionis* adults and cocoons were shipped.

SHIPMENT.—All *B. curculionis* were forwarded to the Insect Identification and Parasite Introduction Laboratory at Moorestown, N. J. Adults were shipped in pint-sized ice-cream cartons loosely filled with excelsior. Streaks of honey on the inside of the cartons served as food. Initial shipments of adults suffered high mortality even though they were usually received within 24 hours. This result was attributed to a lack of moisture which the parasites apparently needed to sustain themselves. Moistening the excelsior before shipping reduced mortality. Cocoons also were shipped in ice-cream cartons with cotton or absorbent tissue as a baffle to prevent injury.

EMERGENCE FROM COCOONS.—Nondiapausing *curculionis* emerged from cocoons shortly after arriving at Moorestown. Cocoons which contained parasites in diapause were held for 3 to 4 months at room temperature (70° to 80° F.) and then placed in a cold room with a temperature of 40°. They were removed the following spring for emergence timed to coincide with the presence of optimum abundance of weevil larvae. Monthly moistening of the cocoons when held at 40° appeared to increase adult emergence. When this procedure was followed, 80% to 90% emergence was obtained.

RELEASES.—A total of 6,086 *B. curculionis* were released—2,833 in 1959, and 3,253 in 1960. Release fields during 1959 were chosen in three states: Delaware, New Jersey, and Virginia. The Virginia field at Onancock, Accomac County, was selected because weevil larvae were present earlier in the year than in the other two States and the earliest available parasites could be released there. Later, as the weevil population became abundant, releases were made in the more northern localities, in Delaware at Dover and Smyrna in Kent County, and at Middletown in New Castle County. New Jersey releases were at Columbus, Mt. Holly, Medford, and Rancocas, all in Burlington County. The 1960 releases were all confined to New Jersey with the exception of one, at Clarksville, Md. Additional release sites in New Jersey included Pemberton, also in Burlington County, and Ringoes in Hunterdon County. All fields in which releases were made were chosen to give the parasite every available opportunity to become established under eastern climatic conditions. In New Jersey alfalfa fields in the Soil Bank Conservation Reserve Program were utilized. These were not sprayed and had the added advantage of not being cut or trimmed until the weevil population had disappeared from the field. In the other states it was understood that the release fields were not to be sprayed, although normal cultural practices were followed.

Table 1.—Recoveries of *Bathyplectes curculionis* parasitizing alfalfa weevil from release fields in three States, 1960.

LOCATION	YEAR OF RELEASE	NO. OF PARASITES RELEASED	NUMBER OF LARVAL SAMPLES TAKEN	DATE OF PARASITE RECOVERIES	TOTAL PARASITES REARED (NO.)
Delaware					
Dover	1959	223	3	April 27, June 9	59
New Jersey					
Mt. Holly	1959	70	7	May 17, May 27, June 7	12
Columbus	1959	493	6	May 17, May 31, June 13	156
Pemberton	1960	646	4	May 12, May 31, June 13	12
Rancocas	1959, 1960	1,105	7	May 10, May 17, May 27, June 7	48
Ringoes	1960	1,517	4	May 10, May 31, June 13	8
Virginia					
Onancock	1959	1,569	2	April 27[a]	2

[a] Field sprayed with malathion in May 1960.

RESULTS AND DISCUSSION.—Limited attempts to recover the parasite were made during 1959. Of all the release fields sampled, only in the one at Mt. Holly, N. J., were any parasite cocoons found, and then only six. Adults emerged from four of the six cocoons the following spring, and these were identified as *curculionis*. The 1960 attempts at recovering *curculionis* were intensified. Each release field was sampled in April and at periodic intervals thereafter until the weevil population had almost disappeared at the end of June or in early July. The sampling process consisted of taking 50 sweeps throughout the field when the weevil population was high (1,000+ larvae). When the population was less than 200 larvae per 50 sweeps, the number of sweeps was increased to 100. The larvae collected were brought back to the laboratory for parasite rearing. The rearing method generally used was that followed in California; however, when only a few weevil larvae were obtained, they were reared in ½-pint ice-cream cartons instead of in cages.

Recoveries from release fields made in 1960 are shown in table 1. The total parasites recovered from each field may not appear impressive, particularly if the sample from Columbus, N. J., that produced 156 cocoons is excepted. Yet it is significant that recoveries were made from the majority of fields where *curculionis* adults were released. These recoveries are especially significant in view of the small number of individuals released (half of which were males) compared with the total host population in the particular release field.

From these recoveries cocoons containing both dia-

pausing and nondiapausing *curculionis* were encountered, which indicated that at least a small second generation was produced in 1960. The recoveries also demonstrated the ability of the parasite to survive in the climate of the eastern United States. Its capacity to spread was indicated by the recovery of two parasite cocoons that were reared from a sample of weevil larvae collected one-half mile north of the Columbus, N. J., release field.

Although it is too soon to state categorically that *curculionis* is permanently established in the eastern United States, certainly it has established a very substantial "beachhead." Granting that it is established, the ultimate effectiveness of the parasite in bringing about a reduction in the alfalfa weevil population will not be known until it has multiplied and become distributed over a much larger area. During 1961 the parasite's progress in the established fields as well as its natural dispersal will be observed. Additional releases are also contemplated should more stock be available.

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Development of Resistance to DDT by the Apple Maggot, *Rhagoletis pomonella*[1]

DAVID E. LEONARD, *The Connecticut Agricultural Experiment Station, New Haven*

ABSTRACT

Tests in dosage series were conducted comparing a population of apple maggot (*Rhagoletis pomonella* (Walsh)) flies from a sprayed orchard with one from unsprayed trees. The tests showed a difference in the two populations, with the flies from the sprayed orchard less susceptible to DDT. The degree of difference was not large. However, both populations exhibited an ability to recover from tremors induced by DDT. More flies from the sprayed orchard were able to recover, but since this recovery also occurred in the population from unsprayed trees, it is suggested that genetic mixture has occurred between populations. Since the susceptibility to DDT of the "original population" of the apple maggot is not known, the level of resistance brought about by the ability to recover from DDT-poisoning cannot be determined.

The possible effect of the recovery mechanism in the field is theorized. The increased tremors may cause the insect to leave the sprayed surfaces. If the affected insect reaches a surface uncontaminated with DDT, recovery is possible.

The apple maggot *Rhagoletis pomonella* (Walsh), Connecticut's most serious apple insect pest (Garman & Townsend 1952), has in recent years become increasingly difficult to control. Since resistance to insecticides has been suggested as a possible reason for this difficulty (Garman 1957), this study was undertaken to determine if the apple maggot is resistant to DDT.

The control of the apple maggot in the field is difficult for several reasons. Proper timing of sprays, use of effective insecticides, and sufficient spray coverage are important. However, climatic factors may increase the longevity of the flies late in the season, when the use of many insecticides will cause unacceptable residues on the fruit. Migration of flies from unsprayed regions also intensifies the problem of control.

METHOD AND MATERIALS.—As Spiller (1958) has pointed out, there is an especially difficult problem of obtaining and sustaining susceptible and resistant insects for laboratory study. This is very evident in regard to the apple maggot. In some orchards, even though adequate control measures have been applied, the per cent of infested fruit remains high, suggesting that insect resistance had developed. Such orchards provide a source of suspected resistant flies. The selection of a known susceptible population of apple maggot is much more difficult. Hoskins (1960), in discussing the detection and degree of development of resistance in an insect in a given region, states that the possibility of an insect becoming resistant is largely controlled by the variation in susceptibility present in the original population. Therefore, the susceptibility of the insect should be determined before the widespread use of the insecticide. There is no such information about the susceptibility of the apple maggot. To establish a basis of comparison for the suspected resistant population, flies were collected from abandoned orchards and trees that had no record of having received syn-

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thetic organic insecticides. Although this population may represent the most susceptible flies available, it cannot be determined how their susceptibility compares with the susceptibility of the "original population" of the apple maggot.

Apple maggot flies were obtained by collecting infested fruit and placing this fruit in screened trays over sand. The mature larvae, after leaving the fruit, dropped to the sand, where they pupated. The puparia were recovered by washing the sand through a screen, and were then placed in a controlled room (27° C., 50% relative humidity) until the flies emerged.

Tests in dosage series utilizing two methods of exposure to DDT were conducted in a controlled room. The first method was by confining flies in petri dishes which had received known amounts of p-p'DDT. The DDT was dissolved in acetone and pipetted into the petri dish. The acetone was allowed to evaporate before the flies were placed in the petri dish. Although the time of exposure was regulated, it was not known how much DDT the flies actually obtained. Topical application tests were adopted, whereby flies received known amounts of p-p'DDT in 1 microliter of acetone applied to the venter of the thorax. Check flies for both types of tests were handled in the same manner as treated flies except they were not exposed to DDT. In the topical application tests, the check flies received 1 microliter of acetone. All four dosage levels were duplicated once, with a total of 20 to 35 flies from 1 to 7 days old used for each dosage level. Flies of both sexes were used. Mortality counts were made 48 hours after exposure. The regression lines were calculated by the method of Bliss (1935). Control mortality was corrected by Abbott's formula. For ease of handling, flies were anesthetized with carbon dioxide, with care taken to avoid overexposure, or chilled in petri dishes over an ice water bath.

Before and after exposure, flies were kept in half-pint paper containers and fed a mixture of honey and water taken up in cellucotton. When kept in this manner, flies normally lived 1 to 2 months in the laboratory.

RESULTS AND DISCUSSION.—The results of the dosage tests comparing a population from a sprayed orchard (T) with a population from unsprayed regions (U) are given in figures 1 and 2. The results, which are shown in figure 1, were obtained with the petri dish method of exposure. The topical application tests, shown in figure 2, are considered more valid, since each fly received a known amount of DDT.

The dosage mortality curves denote a difference in susceptibility to DDT in the two populations, but this difference is not large. However, these data may be of more consequence in the light of observations made during and shortly after the exposure of apple maggot flies to DDT.

The effect of DDT on apple maggot flies is much the same as the effect exhibited by *Periplaneta americana* as depicted by Tobias & Kollros (1946). The first noticeable effect in the apple maggot fly is an increase in erratic movement followed by a hyperextension of the legs. With the ensuing tremors comes a progressive loss of stability, with the insect repeatedly falling on its back. However, some apple maggot flies are able to recover from these effects. Those flies that do not recover are soon unable to right themselves. Paralysis follows, with the legs appearing to be the last region paralyzed. The moribund flies are normally dead within 24 hours.

The ability of some apple maggot flies to recover from the effects of DDT-poisoning was first noted in preliminary petri dish tests, where tremors were the criteria used to regulate exposure time. When all flies were experiencing tremors, they were removed to a clean cage. Even though many flies exhibited severe tremors with almost a complete loss of stability before the termination of exposure, few of these flies were dead after 24 hours. Both populations of flies exhibited this ability to recover from the effects of DDT-poisoning. There were no apparent deleterious aftereffects or decrease in longevity in the flies that recovered.

The mechanism enabling apple maggot flies to recover from the effects of DDT is not completely understood. Preliminary tests on a mixed population of apple maggot flies with Wisconsin Alumni Research Foundation (WARF) antiresistant for DDT indicate a higher mortality obtained when the antiresistant is used. This difference is not so striking as the effect of WARF antiresistant when used on DDT-resistant house flies (*Musca domestica* L.). This indicates that either the apple maggot flies contain a higher level of DDT-ase activity or that another mechanism is also involved.

In the apple maggot, both the suspected resistant and

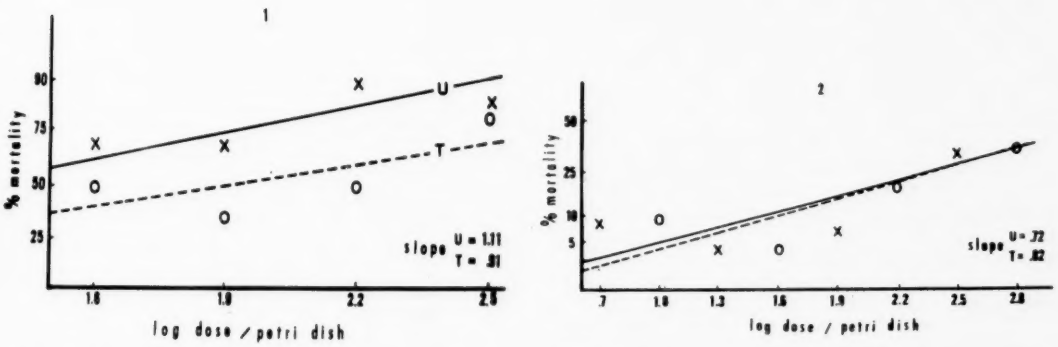

FIG. 1.—Comparison of the susceptibility to DDT of two populations of apple maggot flies. Population T is from a sprayed orchard, and population U is from unsprayed regions.

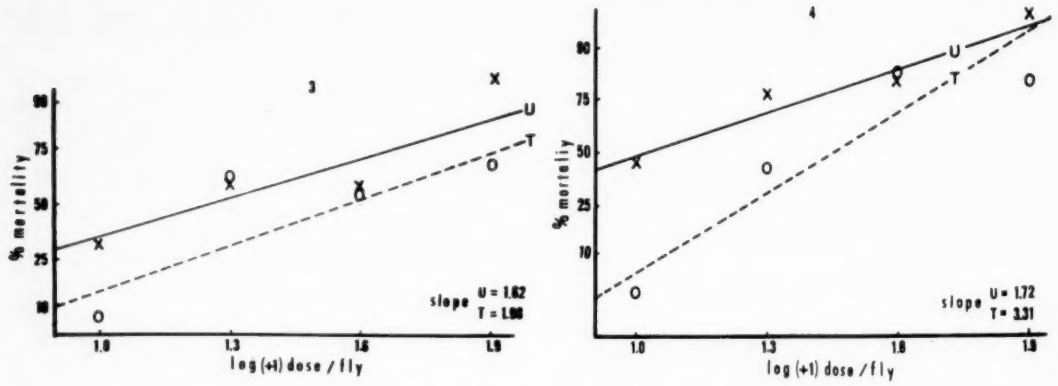

FIG. 2.—Comparison of the same populations of apple maggot flies using topical application method of exposure to DDT.

susceptible populations were able to recover from the effects of DDT-poisoning. Smyth & Roys (1955), studying resistant and susceptible strains of house flies, noted the effects of DDT-poisoning is irreversible in the susceptible strain. The ability of the susceptible apple maggot population to recover from the effects of DDT may be answered in two ways. First, the recovery mechanism may be present in the original population of the apple maggot, in all flies or in a high frequency. Second, flies exposed to the selective action of DDT, migrating to the unsprayed regions, may have caused a mixed genome. This latter alternative seems the more defensible, since migration readily occurs in this species. Therefore, this suspected susceptible population may represent a popu'ation of susceptible, resistant, and hybrid flies. This condition has been shown by Davidson (1956a, b) to occur in *Anopheles gambiae* in Nigeria.

The ability of apple maggot adults to recover from exposure to DDT may suggest a behavioral aspect to the difficulty of control in the field. The effect of DDT causes a progressive loss of stability, and if the flies do not voluntarily leave the sprayed surface of the apple tree, they would fall from this surface as the tremors become more pronounced. If the insect falls on, or flies to a surface uncontaminated with DDT, recovery is possible.

In the field, it seems likely that continued selective pressure of DDT and most likely other related chlorinated hydrocarbons will eliminate those flies least able to recover from the effects of DDT-poisoning. The level of resistance that might be attained is not known.

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Seed Systemics for Control of Aphids on Oats and Barley[1]

Robert F. Harwood and G. W. Bruehl[2]

Abstract

Eleven organophosphorous compounds in carbon formulation were tested as seed systemics for phytotoxicity to barley and oats, and control of aphids on oats. Phorate, Bayer 25198 (*O,O*-dimethyl-*O*-*p*-(methylsulfinyl)phenyl phosphorothioate), Di-Syston® (*O,O*-diethyl *S*-2 (ethylthio)ethyl phosphorodithioate), dimethoate, and phosphamidon were promising. Phorate and Di-Syston did not prevent transmission of barley yellow-dwarf virus to small plots of oats by migrating alate aphids.

Aphids attacking cereals may be of serious consequence because of direct injury or transmission of pathogens. It is often not practical to apply insecticides, particularly under dry-farming, because of a limited margin of profit. It would be desirable to control aphids economically before they build up in large numbers, thus eliminating the expense of applying insecticides by aircraft.

This investigation was initiated to attempt control of aphids transmitting the yellow dwarf virus of cereals. The disease is prevalent annually in barley and oats in Washington west of the Cascade Mountains. In 1958 it almost completely destroyed spring plantings of barley in large areas of eastern Washington.

Seed systemic control of aphid vectors was tested because this method affords best protection during early seedling stages, the period when host plants are most severely damaged by the virus (Oswald & Houston 1953). Furthermore, seed treatment is cheap and fits in with the current practice of slurry treating seed to control cereal smuts.

Three species of aphids were present at Vancouver, Washington, where field evaluations took place. These were the rose grass aphid, *Macrosiphum dirhodum* (Walker); apple grain aphid, *Rhopalosiphum fitchii* (Sanderson); and English grain aphid, *M. granarium* (Kirby). The first two species were most prevalent in early spring. By June, the apple grain aphid had left cereals and the English grain aphid was most prevalent.

Eleven organophosphorus compounds were rather extensively tested as seed systemics. These included four Monsanto compounds that are not further discussed because their chemical structure could not be obtained. It can be stated that these four compounds were either excessively phytotoxic or ineffective in controlling aphids. All but one of the compounds tested were formulated on carbon and were thoroughly mixed by means of a jar roller (Chao & Roberts 1954). The correct amount of carbon-formulated insecticide was placed with 0.01 bushel of seed in a quart jar. Twenty milliliters of an aqueous solution of 1% methyl cellulose was added to each jar as a sticker, and the jar was rolled for 20 minutes. Preliminary information was obtained on phytotoxicity by seed germination tests, followed by exposure to aphids in the greenhouse, and finally by field tests.

Germination tests were performed in damp sand. Shasta oats and Gem barley were the varieties tested. Data given in table 1 show that practically all the insecticides caused some malformation of sprouts. The damage increased with increased dosage. Oats were more susceptible to certain compounds and barley to others.

Table 1.—Effect of organophosphorus seed systemics on germination of Shasta oats and Gem barley. Pullman, Washington. 1959.

Compound	Active Ingredient (oz./bu.)	Normal Sprouts[a] (%)	
		Oats	Barley
Untreated	0	100	93
Bayer 25141[b]	1	97	82
	2	75	64
	4	54	16
Bayer 25198	1	95	87
	2	92	78
	4	65	68
Bayer 29493[c] (Dimethyl 3-methyl-4-methylthio-phenyl thiophosphate)	1	99	84
	2	94	77
	4	94	74
Di-Syston	1	98	86
	2	95	84
	4	88	75
Phorate	1	83	80
	2	52	80
	4	15	62
Dimethoate	1	67	21
	2	32	12
	4	7	0
Shell BAS 3562[d]	1	94	85
	2	84	77
	4	80	74
Phosphamidon	1	88	83
	2	87	83
	4	59	82

[a] Based on an average of two sets of 100 seeds each.
[b] *O,O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate.
[c] *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate.
[d] A full strength liquid formulation (2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) which was inadvertently deleted from further evaluations.

The 4-ounce-per-bushel concentration of seed systemics was tested on oats in the greenhouse, to determine interval of protection from infestation by rose grass aphid and English grain aphid. Five seeds treated with one compound were placed in two 4-inch clay pots (total of 10 seeds) in a rich soil mixture. Heavily infested oats were kept in the same greenhouse section. Thus, the treated plants were continuously exposed to infestation by migrating alate aphids. In addition, portions of these heavily infested plants were placed every 3 days or less upon the experimental pots. Results are summarized in table 2. All compounds prevented aphids from actually killing the plants. Phorate and Di-Syston® (*O,O*-diethyl *S*-2(ethyl-

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[2] Associate entomologist and associate plant pathologist, respectively, Washington State University, Pullman, Washington. The assistance of Mr. Richard Welton during germination trials and greenhouse evaluations is appreciated.

Table 2.—Effectiveness of 4 oz./bu. rates of seed systemics in preventing infestation of Shasta oats by rose grass and English grain aphids. Pullman, Washington. April–May, 1959.

Compound	Percentage Plants Emerged	Days After Planting[a]								
		14	16	18	21	28	35	41	43	49
Control	60	0[a]	*	*	*	*	X			
Bayer 25141	40	–	–	–	+	+	*	*	*	*
Bayer 25198	40	0	–	–	–	+	*	*	*	*
Bayer 29493	40	–	–	*	*	*	*	*	*	*
Di-Syston	10	0	–	0	0	0	–	+	+	*
Phorate	40	0	–	0	0	0	–	+	+	*
Dimethoate	90	0	–	–	–	+	*	*	*	*
Phosphamidon	60[b]	–	–	–	+	+	*	*	*	*

[a] 0, no infestation; –, adults, no young; +, adults, few young; *, adults and numerous young; X, plant killed by feeding of aphids.
[b] Based on similar tests for which aphid infestations were not obtained.

thio)ethyl phosphorodithioate) provided nearly complete protection for 28 days under these conditions, and prevented heavy infestation through 43 days.

Shasta oats were treated with 4 ounces actual insecticide per bushel. One short row of each treatment, plus untreated control was hand planted at Vancouver, Washington, in two locations. Approximately 35 seeds were planted in each row. Plots were established on April 7, 1959, and evaluated 24 days later. The plantings were located between heavily infested fall plantings of mixed cereals (wheat, oats, barley) and were therefore subject to exceptionally high attack. The results are summarized in table 3. Di-Syston, phorate and dimethoate provided considerable control, with only slight evidence of stunting or distortion of plants.

Field plots of Shasta oats were established at Vancouver, Washington on May 1, 1959. These plantings were started at this late date to ensure an adequate infestation of aphid vectors while the seedlings were still small and susceptible. Each treatment consisted of an unreplicated plot an acre or more in size. Phorate and Di-Syston, the systemics tested, were applied at rates of 4 ounces of actual toxicant per bushel.

Aphid infestation began at emergence, was general, uniform, and a 100% infection with yellow dwarf was obvious by the jointing stage. Evaluations were made by carefully collecting 12 main stems from near the center of each block. Total counts of aphids were made, and an average count per plant calculated. The three aphid

Table 3.—Control of three species of aphids on Shasta oats by seed systemics at 4 ounces actual toxicant per bushel. Vancouver, Washington. April–May, 1959.

Compound	Total		Aphids /Plant[a]	Per Cent of Full Stand	Per Cent Control of Aphids /Plant
	Plants	Aphids			
Untreated	65	1688	25.7	100	0
Bayer 25141	32	156	4.9	49	81
Bayer 25198	53	416	7.7	70	81
Bayer 29493	37	650	17.6	57	32
Di-Syston	41	131	3.2	63	88
Phorate	45	38	.8	69	96
Dimethoate	48	114	2.4	74	91
Phosphamidon	67	665	9.9	103	62

[a] 24 days after planting.

Table 4.—Control of aphid vectors of barley yellow dwarf virus by seed systemics. Vancouver, Washington. May–June, 1959.

Treatment	Aphids per Plant		Yield (bu./acre)
	10 Days[a]	21 Days	
Control (no treatment)	30.8	12.4	24.4
Control (no treatment)	24.0	15.5	35.6
Control (aqueous methyl cellulose only)	40.1	20.6	33.7
Phorate 4 oz./bu.	6.3	22.5	28.6
Di-Syston 4 oz./bu.	3.0	17.2	39.6

[a] Days after emergence.

species noted earlier were present at the first evaluation, but no apple grain aphids remained at the second count. The results in table 4 indicate that control evident in the first 10 days was negated by 21 days. Yield data indicate that no significant control of barley yellow dwarf was achieved.

Conclusions.—From the data at hand it may be concluded that: 1) Several seed systemics markedly reduce aphids in early growth stages of oats. 2) Aphid control was frequently associated with excessive phytotoxicity. 3) Under heavy field infestation, even the most promising systemics, while achieving considerable control of aphids, were unable to prevent the transmission of barley yellow dwarf virus to oats. However, it should be noted that relatively small plots were involved, which could easily be covered by migrating alate aphids. 4) On the basis of aphid control and minimal phytotoxicity, of 11 compounds tested, phorate, Bayer 25198 (*O,O*-dimethyl-*O*-*p*-(methylsulfinyl)phenyl phosphorothioate), Di-Syston, dimethoate, and phosphamidon showed promise.

Discussion.—The findings related here agree with those of Pizarro & Arny (1957, 1958) who noted that soil drenches, foliar sprays and seed treatment with demeton rendered oats toxic to apple grain aphids for prolonged periods, but did not prevent infection with barley yellow dwarf virus by migrants. Dickason et al. (1960) in Oregon also found applications of granules of phorate and Di-Syston achieved early control of aphids on barley, but did not prevent complete infection with barley yellow dwarf virus. In marked contrast, Caldwell et al. (1959) achieved spectacular control of this same disease on oats in Indiana, with a foliar spray of dimethoate. These results suggest that seed treatments show promise for reducing populations of aphids on cereals, but do not destroy aphids rapidly enough to prevent acquisition of readily transmitted diseases from infected migrants.

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Natural Control of Aphid Populations on Cole Crops[1]

DAVID PIMENTEL,[2] *Department of Entomology, Cornell University, Ithaca, N. Y.*

ABSTRACT

The factors involved in the natural control of the most numerous aphids on cole crops were studied. During the 1957 season the cabbage aphid (*Brevicoryne brassicae* (L.)) was the most abundant aphid followed by the green peach aphid (*Myzus persicae* (Sulzer)), whereas the turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis) was least abundant. In 1958 the order of abundance was: the green peach aphid, turnip aphid, and cabbage aphid. A parasite, *Praon* sp., attacked only *M. persicae*. *Diaeretus rapae* (McIntosh) and *Empusa aphidis* Hoffman attacked all three aphids. The ratio of parasitism was greatest in the most dense aphid population and smallest in the sparse population. Coccinellids of the following species destroyed aphids: *Hippodamia convergens* Guérin-Méneville, *Ceratomegilla maculata fuscilabris* De Geer, and *Coccinella transversoguttata* Faldermann. Syrphids attacking the aphids were: *Sphaerophoria cyclindrica* Say, *S. menthastri* (L.), *S. robusta* (Curran), and *Mesogramma marginata* Say.

The cabbage aphid (*Brevicoryne brassicae* (L.)), turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis)) and green peach aphid (*Myzus persicae* (Sulzer)) are the most important aphids attacking cole crops. In controlling these pests, naturally or chemically, especially from the point of view of "integrated control" (Stern et al. 1959), the community ecology of these pests must be understood. Therefore, the objective of this investigation was to study the ecological relationship which exists between *B. brassicae*, *M. persicae* and *R. psuedobrassicae*.

The theories of natural control for these aphid species are varied. Evans (1938) found the reproductive rate of *B. brassicae* was positively correlated with the nitrogen content of the host plant and in particular with the protein content, and he pointed out that this factor played an important role in aphid outbreaks in the fall. Gourlay (1930) concluded that the small braconid wasp (*Diaeretus rapae* (McIntosh)) was of primary importance in *B. brassicae* control. Prethebridge & Mellor (1936), however, reported that under hot dry conditions the many parasites and predators could not control *B. brassicae*. These workers further suggested that control was possible only when the aphid was adversely affected by cool-wet conditions (Prethebridge & Wright 1938). Natural enemies of *B. brassicae* were listed by Markkula (1953) in order of greatest importance as braconid parasites, coccinellids, syrphids, and other enemies. But George (1957) claimed that syrphids were the most effective control agent.

In his investigation of the ecology of *R. pseudobrassicae*, Paddock (1915) felt that the two braconid wasps, *D. rapae* and *Lysiphlebus testaceipes* Cresson, were the primary enemies of *R. pseudobrassicae* and the coccinellids were of lesser importance in aphid control. Davis & Satterthwait (1916) reported that the parasitic wasps *D. rapae* and *Pachyneuron micans* Howard were the primary agents of natural control for *R. pseudobrassicae*.

Populations of *M. persicae* have been studied carefully on many crops but its natural control on cole crops has not been investigated. Because *M. persicae* overwintered on cabbage, it attacked spring set cabbages and other plants earlier than normal since it did not return to its primary Rosacae hosts (Davies 1938). Broadbent (1949) found that microclimatic factors influenced the activity of the alate forms of *M. persicae* on cabbage.

METHODS.—The study of the ecological relationship of the three species of aphids was carried out during 1957 and 1958 in plots 75 feet by 100 feet. In the summer of 1957 the following cole varieties were planted: broccoli, Brussels sprouts, cabbage, collards, and kale. During 1958 only collards were used, because the broad, flat leaves of collards facilitated counting. The plants were 18 inches apart in rows 100-feet long, which were 3 feet apart. The five row-blocks of each variety were set random to one another. The plots were fertilized with a 5–10–5 mixture at a rate of 2,000 pounds per acre and they were limed at a similar rate. The plots were maintained weed free.

The insect species samples and the stages counted on each of the 100 plants sampled weekly are given below:
Aphids—The *B. brassicae*, *R. pseudobrassicae*, and *M. persicae* were all counted as adults.
Parasites—All hymenopteran parasites were counted as so many parasitic attacks on the aphids. Those aphids parasitized and attached to the plant were counted as parasitized. Aphids from which parasites had emerged were not counted. When more than 100 parasitized aphids were present on one plant, 100 were taken as a sample, otherwise all parasitized aphids were taken to the laboratory for holding to determine the parasites and their ratios.
Predators—The Coccinellidae were counted as adults; the Syrphidae were counted as pupae.

In 1957 counts were initiated May 28th, in 1958, June 3rd, and counts during both seasons were made weekly for 15 weeks. Weekly, 100 plants were selected systematically to insure a representative sample of the plot and to avoid bias in the choice of individual plants.

RESULTS.—During 1957 *B. brassicae* was the most

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[2] I thank Professor John A. Weidhaas for carefully reviewing this manuscript and for his helpful suggestions.

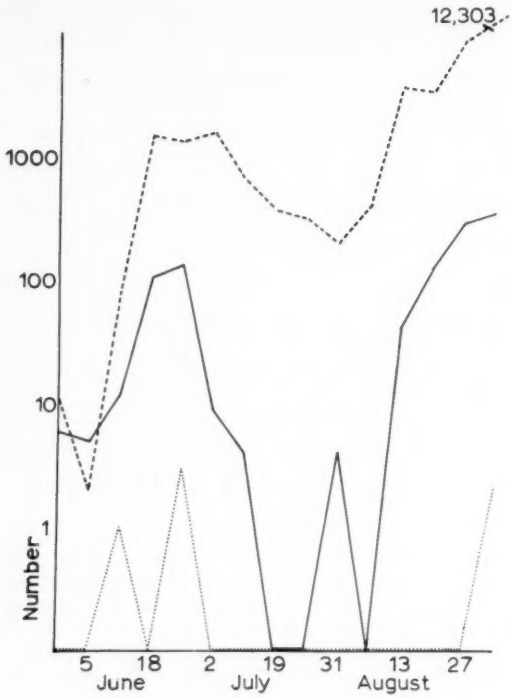

FIG. 1.—The number (log) of *B. brassicae* (– – – –), *M. persicae* (————), and *R. pseudobrassicae* (·····) per 100 plants during 1957.

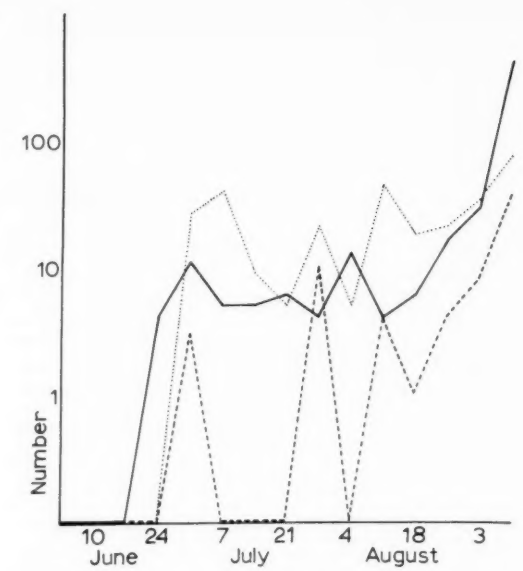

FIG. 2.—The number (log) of *B. brassicae* (– – – –), *M. persicae* (————) and *R. pseudobrassicae* (·····) per 100 plants during 1958.

abundant aphid with an average weekly density of 2,323.6 per 100 plants, *M. persicae* followed with an average density of 71.8, and *R. pseudobrassicae* was least abundant with a density of 0.4 (fig. 1). In 1958 the community picture changed, all the aphid populations declined except for *R. pseudobrassicae*. *M. persicae* was then the most abundant aphid with an average density of 33.7, followed in order by *R. pseudobrassicae* with an average density of 19.7 and *B. brassicae* with an average density of 4.5 (fig. 2). The density of *B. brassicae* declined most sharply followed by *M. persicae*. *R. pseudobrassicae* increased 10-fold over the 1957 density.

Rapid changes in the aphid populations occurred during both seasons (figs. 1 and 2). Aphid populations increased rapidly during late June and also during early August. Maximum density was reached at the close of the season. The cool conditions existing both in spring and fall apparently favored the aphids. During the spring and fall, it was probably cool enough to inactivate the aphid parasites, but still sufficiently warm for the aphids to be reproductively active (Paddock 1915). The high nitrogen levels in the plants in the fall may also have favored the aphids and especially *B. brassicae* (Evans 1938).

Although in 1957 parasite densities followed the trends of the aphids, they generally lagged behind (fig. 3). This was expected because the parasites depend upon the aphids for their maintenance. Both *D. rapae* and *Praon* sp. are primary parasites of the aphids whereas both *Charips brassicae* (Ashmead) and *Asaphes fletcheri* (Craw-

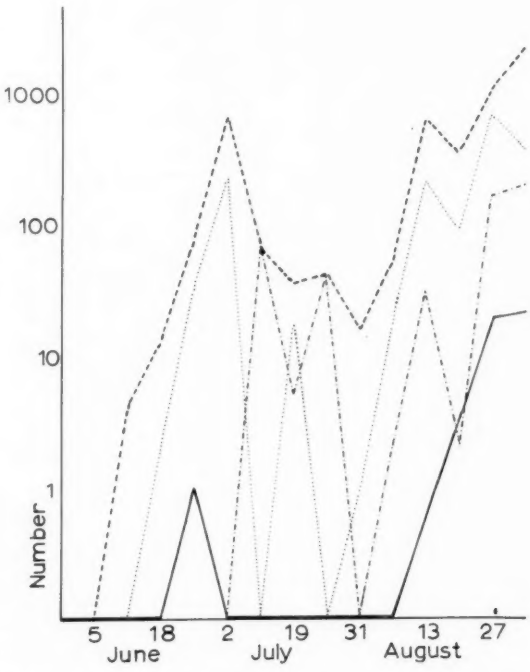

FIG. 3.—The number (log) of *D. rapae* (·····), *C. brassicae* (– – – –), and *A. fletcheri* (– · – · –) in *B. brassicae* and number of *Praon* sp. (————) in *M. persicae* per 100 plants during 1957.

ford) are secondary parasites. Note that in total numbers the secondary parasites were more abundant than the primary parasites during 1957 (fig. 3) and they were not far behind this during 1958 (fig. 4). For example, in 1957 *C. brassicae* averaged 316.5 whereas *D. rapae* averaged 102.9. This condition is the opposite of what would be expected, that is, generally there are more primary parasites than secondary parasites because the secondary parasites depend upon the primary parasites for support. One possible explanation is that *D. rapae* has a high fecundity, which supported not only large numbers of *C. brassicae* and *A. fletcheri* but produced a sufficient number of itself to maintain its species.

In 1957, *B. brassicae* was severely attacked by *D. rapae* which had an average density of 102.9 (fig. 3). *Praon* sp., with an average density of 28.0, attacked *M. persicae*. Only one *B. brassicae* was observed infected with the fungus and this was on June 18, 1957. During 1958, *D. rapae* had an average density of only 2.3 in the aphid, *M. persicae* (fig. 4). In the next season, only two *D. rapae* were found in the *B. brassicae* population. Fungus, (*Empusa aphidis* Hoffman) infestations in *M. persicae* averaged 1.5 weekly (fig. 4). During 1958 no *Praon* sp. were detected. The heaviest parasitism during the season was in the most abundant aphid population, *M. persicae*.

In both years the heaviest parasitism in the aphid populations occurred late in the season, when aphid populations were most abundant (figs. 1, 2, 3, and 4). On September 5, 1957, the densest *B. brassicae* population was 18.2% parasitized, 5.8% of *M. persicae* were parasitized, and none of the *R. pseudobrassicae* population were parasitized. In the next season, on September 9, 1958, the highest *M. persicae* population was 17.9% parasitized; the lowest *B. brassicae* population was 16.2% parasitized; and no parasitism was observed in the *R. pseudobrassicae* population on this day.

Thus, in both seasons parasitism was heaviest in the most dense aphid populations, except for the *R. pseudobrassicae* population during the 1958 season. This trend would be expected if the parasites were species specific, but, except for possibly *Praon* sp., they were not species specific in the *B. oleracea* community. *D. rapae* and the fungus (*E. aphidis*) attacked all three species of aphids. Although in this investigation *R. pseudobrassicae* was not parasitized with *D. rapae*, parasitism has been noted in other studies (Spencer 1926, Paddock 1915, Pimentel 1961). Generally, increased host density leads to increased parasitism (Salt 1934), and any increase in the total aphid population should lead to an equal increase in parasitism in all aphid species. This did not happen, however, and the reason is evident—the aphid species populations were not randomly mixed. That is, aphids normally exist on plants in single-species colonies. The larger the total population the larger the colonies and a greater number of colonies are present. *D. rapae* searching, as expected, would locate the more numerous and larger colonies more often and a higher rate of parasitism would result in these colonies.

D. rapae did not seem to prefer one aphid species over another. The writer suspects that a difference in the preference of *D. rapae* for *B. brassicae*, *R. pseudobrassicae*, and *M. persicae* does exist. The density-searching relationship probably plays the most important role. That is,

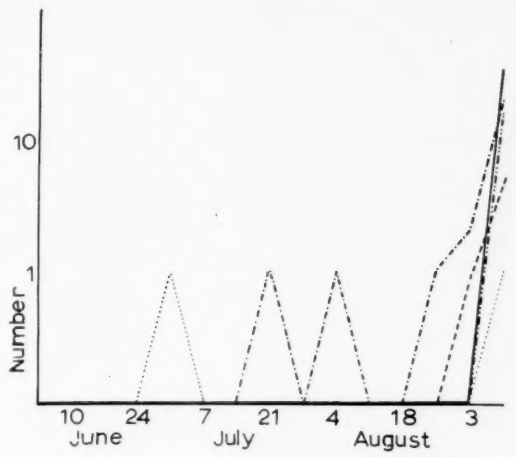

FIG. 4.—The number (log) of *D. rapae* (·····), and *C. brassicae* (– – – –) in *B. brassicae* and *D. rapae* (————), *C. brassicae* (·····), and *E. aphidis* (–·–·–·) in *M. persicae* per 100 plants during 1958.

if *D. rapae* is given an equal choice it might prefer one aphid species to another but when an aphid species is abundant, opportunity for contact is increased so the greatest parasitism occurs in the most abundant species.

Predaceous species attacking the aphids were principally coccinellids and syrphids. In 1957 coccinellids of the following species, in order of abundance, were: *Hippodamia convergens* Guérin-Méneville, *Ceratomegilla maculata fuscilabris* De Geer, and *Coccinella transversoguttata* Faldermann. In 1958 the following coccinellids in order of abundance were: *C. maculata fuscilabris*, and *C. transversoguttata*. The syrphids in order of abundance during 1957 were: *Sphaerophoria cylindrica* Say, *S. menthastri* (L.), and *S. robusta* (Curran). In 1958 the order of abundance was: *Mesogramma marginata* Say, *S. cylindrica*, and *S. robusta*. The average density of the coccinellids during 1957 was 7.0, whereas the syrphids averaged 6.0 (fig. 5). During 1958 the coccinellids averaged 0.5 and the syrphids averaged 0.8 (fig. 6). The ratio of coccinellids and syrphids to aphids in 1957 was 1 predator to 182 aphids, and in 1958 1 predator to 46 aphids. No particular preference by any species of predator for any one species of aphid was observed. Although predator densities were lower in 1958 the ratio of predators to aphids was higher.

The most effective control agent of aphids associated with the cole crops apparently varies according to the environmental conditions; some workers felt that the parasites were the most effective (Gourlay 1930, Paddock 1915, Davis & Satterthwait 1916); but Markkula (1953) reported that syrphids were not effective; and Prethebridge & Mellor (1936) and Prethebridge & Wright (1938) claimed weather conditions were most effective. In this study, during 1957, the ratio was 1 parasite to every 6 aphids and this was greater than the ratio 1 to 182 recorded for the predators for the same year. During 1958 the ratio of 1 parasite to 15 aphids was greater than the predator ratio of 1 to 46. From these data, however, it is impossible to say whether either parasite or predator was

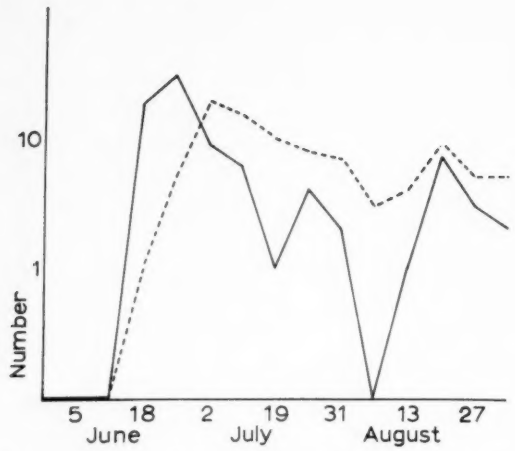

FIG. 5.—The number (log) of coccinellids (————) and syrphids (– – – – –) per 100 plants during 1957.

the more effective for aphid control. Coccinellids and syrphids have been observed to destroy from 5 to 60 aphids per day, but the parasites attack a great many aphids and they out numbered the predators between 3 and 20 times. Another important factor is that the parasites reproduce in about 14 days—or in about one-half the time required by predators. In conclusion, a careful study would be needed to determine which natural enemy was more effective and how all these relationships are influenced by weather.

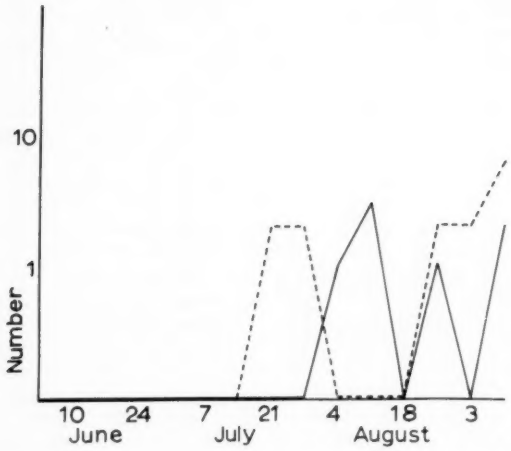

FIG. 6.—The number (log) of coccinellids (————) and syrphids (– – – – –) per 100 plants during 1958.

ECOLOGICAL SIGNIFICANCE.—That the parasite, *D. rapae*, attacked most frequently the densest aphid competitor, demonstrates how interspecific competing species can be maintained together, even though one of the competitors can eliminate the other under certain conditions. Under laboratory conditions favoring one particular aphid species and without parasite attack, one aphid species, *B. brassicae*, *M. persicae*, or *R. pseudobrassicae*, will eliminate its competitor (Pimentel unpublished data). In the natural *B. oleracea* community, however, the winning competitor received the greatest control pressure from the parasite population, thus forcing both species to live together in regulated balance.

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Natural Control of Caterpillar Populations on Cole Crops[1]

DAVID PIMENTEL, *Department of Entomology, Cornell University, Ithaca, N. Y.*

ABSTRACT

In the region of Ithaca, N. Y., both *Pieris rapae* (L.), the imported cabbageworm, and *Plutella maculipennis* (Curtis), the diamondback moth, were more abundant on cole plants than *Trichoplusia ni* (Hübner), the cabbage looper. By weight the order of abundance was *P. rapae*, *P. maculipennis*, and *T. ni*. All three caterpillars were heavily parasitized by their respective parasite complexes, and this aided greatly in controlling the pests. The species of parasite most important one season was not necessarily the most abundant one the following season. Weather and density of caterpillars probably played an important role in determining the abundance of the parasites the year following. Predators contributed to the control of the caterpillars, but their quantitative effect could not be determined in the present design. Many factors determined when during the season the three caterpillars were abundant, and because of this it is difficult without many years of study to predict when the caterpillars will be abundant during any one season.

The most abundant caterpillars attacking cole crops are the imported cabbageworm (*Pieris rapae* (L.)), the diamondback moth (*Plutella maculipennis* (Curtis)), and the cabbage looper (*Trichoplusia ni* (Hübner)). In several parts of the world, studies have been made on the ecology and natural control of these three caterpillars. A comprehensive study of *P. rapae* was made in England by Moss (1933) and Richards (1940), and in New Zealand by Muggeridge (1943). *P. maculipennis* was investigated by Ullyett (1947) in South Africa and by Harcourt (1954) in Canada. The ecology of *T. ni* was studied by McKinney (1944); and the virus-looper association was investigated by Jaques (1960). All the investigators agreed in general that although parasites played an important role in the natural control of all the caterpillars, the relative abundance of the caterpillars, and the species of parasites most important in their control, differed from region to region.

Although all three species of caterpillars are found in the region of Ithaca, N.Y., they are not equally abundant: in addition, the species of their parasites and their relative abundance is not known. The aim of this study was to investigate the ecological relationship existing between the caterpillars and their parasites and also to determine how these enemies contributed to the natural control of the caterpillars in the *Brassica oleracea* L. community.

METHODS.—The study was carried out during 1957 and 1958 in plots 75 feet by 100 feet. In the summer of 1957 the following cole varieties were planted; broccoli, Brussels sprouts, cabbage, collards, and kale. During 1958 collards were used because the broad, flat leaves facilitated insect counting. The plants were in 100-foot-rows placed 3 feet apart, and they were spaced 18 inches in the row. The five-row-blocks of each variety planted in 1957 were set random to one another.

The plots were fertilized with a 5–10–5 mixture at a rate of 2,000 pounds per acre, and they were limed at a similar rate. The plots were maintained weed-free.

The insect species sampled and the stages counted are given below:

Lepidopterons—*P. rapae*, *P. maculipennis*, and *T. ni* were all counted in the last larval stage.

Parasites—*Apanteles glomeratus* (L.), *Phryxe vulgaris* (Fallén), *Horogenes* sp., *Microplitis plutellae* Muesebeck, and *Copidosoma truncatellum* (Dalman) were all counted as so many parasitic attacks on the caterpillars. Those caterpillars counted in the field were taken to the laboratory and held to determine rates of parasitism. Viruses, bacteria, and fungi were counted as so many individual infections of the caterpillars.

Predators—Coccinellids, syrphids, hemipterans, chrysopids, and birds are included in this category. The coccinellids, hemipterans, and chrysopids were counted in the adult stage; the syrphids were counted in the pupal stage. Birds were not counted because there was not a reliable means of estimating their density.

Counts in 1957 were initiated May 28th and in 1958 June 3rd, and in both seasons they were continued for 14 weeks. Once each week 100 plants were sampled in the plot. The plants were selected systematically to insure a representative sample of the plot and to avoid bias in the choice of individual plants.

RESULTS.—During 1957 the order of abundance of the three caterpillars on the 1,500 *B. oleracea* plants examined was as follows: 105 *P. rapae*, 81 *P. maculipennis*, and 7 *T. ni* (figs., 1, 3, and 5). During 1958 the abundance of the caterpillars was: 103 *P. rapae*, 157 *P. maculipennis* and 15 *T. ni* (figs. 2, 4, and 6). The number of any of these species is not large, but the totals are reliable, because 1,500 plants were sampled and the density figures were measured to have an error of less than 10%. From these density data, little change occurred in the density of *P. rapae* from 1957 to 1958, however, significant changes took place in the *P. maculipennis* and *T. ni* populations. The populations of both *P. maculipennis* and *T. ni* approximately doubled. When the densities of *P. rapae* and *P. maculipennis* were averaged for both seasons, there was apparently little difference in the number of individuals of these species in the community. Based on total weight, however, *P. rapae* were greater than *P. maculipennis*. *T. ni* was the least abundant whether the comparison was based on density or weight.

The three caterpillars are known to have different densities in northern and southern North America. In Ontario, Canada, Harcourt (1954) found little overall difference in the abundance of *P. rapae* and *P. maculipennis* on cabbage; on early cabbage *P. rapae* was more abundant whereas on late cabbage *P. maculipennis* was more abundant. *T. ni* was scarce on both early and late cabbage. The present results from the Ithaca region agree favorably with those of Harcourt. This may be due, in part, to the fact that the climate of Ithaca and Ottawa is quite similar. In the warm South, however, the relative

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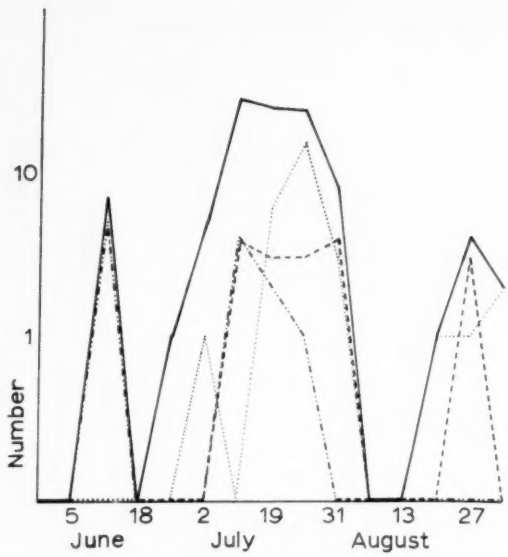

FIG. 1.—The number (log) of *P. rapae* (————), virus (·····), *A. glomeratus* (– – – –), and *P. vulgaris* (–·–·–·–) per 100 plants during 1957.

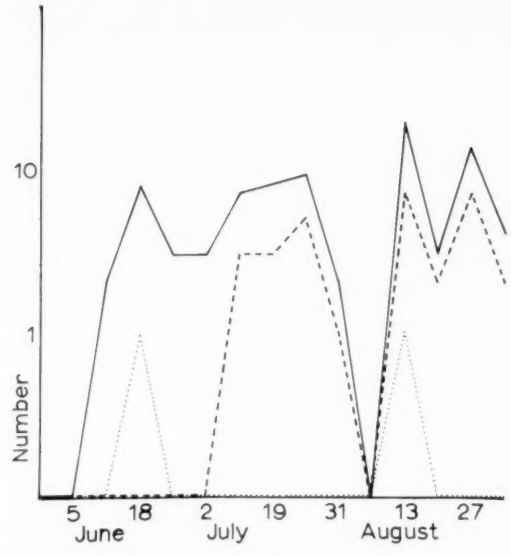

FIG. 3.—The number (log) of *P. maculipennis* (————), *Horogenes* (– – – –), and *M. plutellae* (·····) per 100 plants during 1957.

abundance of the caterpillars is quite different from that found in the North. Harrison & Brubaker (1943), working in Louisiana, report that *T. ni* was the most abundant caterpillar, followed in order by *P. rapae* and *P. maculipennis*.

The peak density for the three species occurred at different times during the season. *P. rapae* during 1957 hit its peak density in the middle of July while in 1958 the peak occurred in late August (figs. 1 and 2). The density of *P. maculipennis* in 1957 reached its peak in mid-June and in 1958 in late June; the densities continued to exist at the high level for the remainder of the season (figs. 3

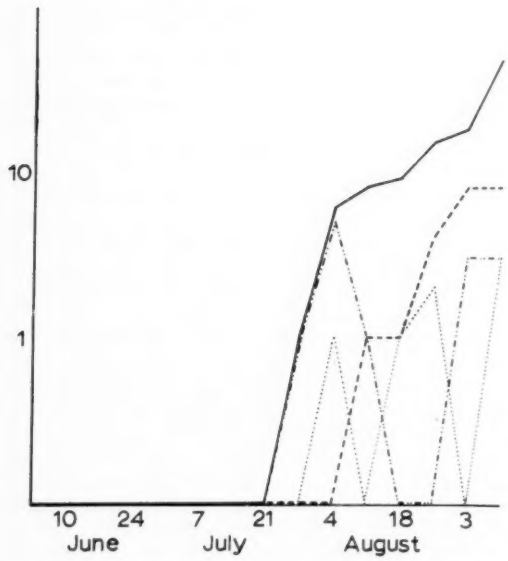

FIG. 2.—The number (log) of *P. rapae* (————), virus (·····), *A. glomeratus* (– – – –), and *P. vulgaris* (–·–·–·–) per 100 plants during 1958.

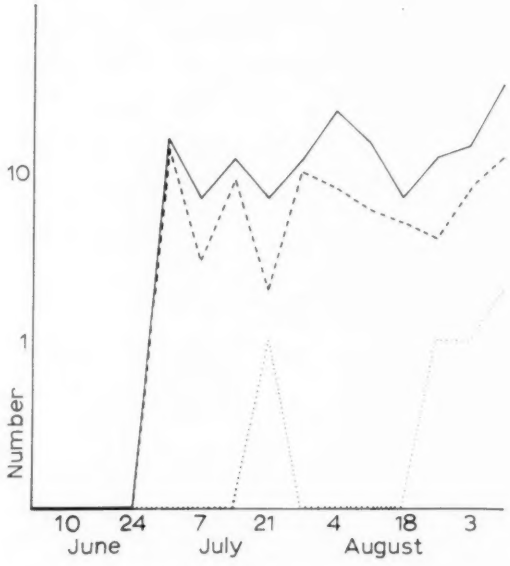

FIG. 4.—The number (log) of *P. maculipennis* (————), *Horogenes* (– – – –), and *M. plutellae* (·····) per 100 plants during 1958.

and 4). These results with *P. rapae* and *P. maculipennis* do not agree with those of Harcourt (1954), who reported that *P. rapae* reached a peak on early cabbage before *P. maculipennis*. The time required for any of these caterpillars to reach peak densities depends upon such diverse factors as weather, parasites, and predators. Thus, the densities of these species would have to be followed for many years to obtain an estimate of yearly time of peak density.

The climate and presence of virus probably have considerable influence on *T. ni* reaching a peak density late in the season (figs. 5 and 6). The nuclear polyhedral virus (*Borrelinavirus* sp.) is highly infective and pathogenic to *T. ni* (Jaques 1960) and was found to be one of the prime factors limiting the density of *T. ni* in the Ithaca region. In addition, *T. ni* is near the limits of its range in this region. The species does best in the subtropics as indicated by the results of Harrison & Brubaker (1943).

Parasitism was generally high in the caterpillar populations. A total of 55% of the *P. rapae* population was parasitized during 1957 and 48% in 1958 (figs. 1 and 2). During the first season *Paillotellavirus pieris* (Paillot) was the principal control agent, destroying 28% of the *P. rapae* caterpillars. *Apanteles glomeratus* parasitized 16% and *Phryex vulgaris* 11% of the *P. rapae* population. In 1958 only 12% were destroyed by the virus; 21% were parasitized by *A. glomeratus* and 14% were parasitized by *P. vulgaris*. The change in parasitism from 1957 to the 1958 season was principally a decrease in virus infections and an increase in *A. glomeratus* parasitism.

Moss (1933) in England found that 60% of *P. rapae* larvae were destroyed by disease (viral and bacterial infections), 34% by *A. glomeratus*, 2% by disease in the pupae, and less than 1% by *Pteromalus puparum* (L.). Working in the same region, Richards (1940) reported that 50% of *P. rapae* larvae were destroyed by disease, sterility, predators, climate, et cetera. Further he found *A. glomeratus* and *Apanteles rubecula* (Hal.) together destroyed 20%, *P. vulgaris* and *Phryex nemea* (Mg.) destroyed 3%, and 1% of the pupae were destroyed by *P. puparum*. Only limited agreement exists between

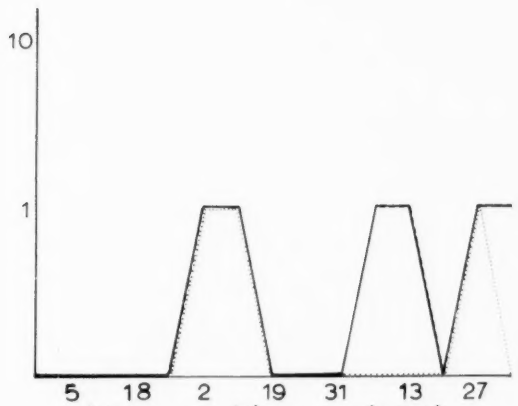

Fig. 5.—The number (log) of *T. ni* (————) and virus (· · · · · · · · ·) per 100 plants during 1957.

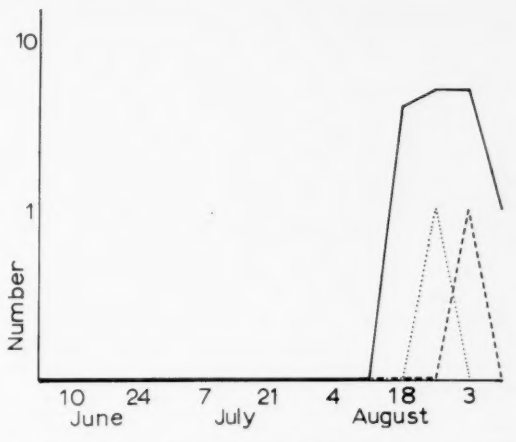

Fig. 6.—The number (log) of *T. ni* (————), virus (· · · · · · · · ·), and *C. truncatellum* (– – – – –) per 100 plants during 1958.

Moss, Richards, and the present study and to attempt any explanation for the discrepancies would be pure speculation.

A total of 40% of the *P. maculipennis* populations was parasitized during 1957 when the population was low (81 individuals) (fig. 3). With a near doubling of the density (157 individuals) of the *P. maculipennis* population in 1958, total parasitism rose to 55% (fig. 4). During 1957, *Horogenes* sp. parasitized 36%, and *M. plutellae* parasitized only 2%. The next season *Horogenes* sp. parasitism in *P. maculipennis* increased significantly to 52%, whereas *M. plutellae* increased to 3%. Ullyett's (1947) work in South Africa showed that between 10% and 70% of *P. maculipennis* larvae were destroyed by parisitism and that *Horogenes* sp. was one of the important parasites. In Canada, Harcourt (1954) found that 25% of *P. maculipennis* larvae were parasitized with *Horogenes* sp., and 11% were parasitized with *Diadromus plutellae* (Ashm.). There *M. plutellae* were also present, but parasitism by this species was rare. The only agreement between the results of Harcourt and this study is that *Horogenes* sp. was the most important parasite.

In 1957 only one parasite, the virus, attacked *T. ni*, and this destroyed 40% of the population (fig. 5). During 1958 only a total of 14% of the *T. ni* population was detroyed, 7% by virus and 7% by *C. truncatellum* (fig. 6). In Arizona, McKinney (1944) reported that a disease (probably virus) was rare in *T. ni*; however, egg parasitism ranged from 0% to 47%, and larval parasitism ranged from 20% to 100%. The tachinid *Voria ruralis* (Fall.) was the most important parasite. Apparently climate plays an important role in the effectiveness of the virus and parasitism; the virus is exceptionally pathogenic for *T. ni* in the Ithaca region.

The various insect predators observed attacking the first and second instars of these caterpillars were: Coccinellidae—*Ceratomegilla maculata fuscilabris* De Geer, *Coccinella transversoguttata* Fald., *Hippodamia convergens* Guér.; Syrphidae—*Sphaerophoria cyclindrica* Say, *S. menthastri* (L.), *S. robusta* (Curran), *Mesogramma margi-*

nata Say; Chrysopidae—*Chrysopa* spp. None of these predators were abundant, and those that were present fed principally on the abundant aphid population (Pimentel 1961b). The average density of the coccinellids during 1957 was 7.0, whereas the syrphids averaged 6.0 weekly per 100 plants. During 1958 the coccinellids averaged 0.5, and the syrphids averaged 0.8 (See figs. 5 and 6 in Pimentel 1961b). Probably no more than 2% to 3% of the caterpillar population was destroyed by these predators. Spiders were relatively abundant in the plots. The average density for 1957 was 149, and for 1958 the density was 167. The species were not determined in sampling, however, a study of the species was made and are listed in another paper (Pimentel 1961a). The number of caterpillars destroyed by spiders was not determined quantitatively, but qualitatively they appear to be an effective control agent.

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Evaluation of Insecticides and Sampling Methods Against the Apple Aphid, *Aphis pomi*[1]

HAROLD F. MADSEN, PETER H. WESTIGARD and LOUIS A. FALCON, *University of California, Berkeley*

ABSTRACT

Studies were conducted in 1959 and 1960 to determine an economic level of infestation for the apple aphid (*Aphis pomi* De Geer) and to develop adequate sampling methods for insecticide evaluations.

The 1959 data showed that sampling from three areas on a shoot, the terminal, central, and basal portions, gave a better indication of aphid numbers than a sample restricted to the terminal leaves. When aphids were present on the central portion of a growing shoot, significant amounts of honeydew were present on the fruit at harvest. Dimethoate in a two-treatment schedule and Diazinon®(*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiosphosphate) in a three-treatment schedule provided adequate control.

The 1960 data confirmed the results obtained in 1959 and in addition indicated that the length of time aphids remain at a high level influences the amount of honeydew present on the fruit at harvest. Dimethoate and Stauffer R-2968 (*O,O*-diethyl dithiophosphorylethyl carbamate) gave good control in the 1960 plots, but Bayer 30911 (methyl-*O*-methyl *O*-2,4-dichlorophenyl phosphonothioate) and granulated phorate were not effective.

Conclusions from the two-year studies were that leaf samples from the central portion of a shoot would indicate if a potentially injurious aphid population is present. If the leaves from this area are infested, the fruit will show damage by honeydew at harvest. The precise number of aphids per leaf is not so important as the distribution of the aphids on a growing shoot.

The apple aphid, *Aphis pomi* De Geer, is the most common aphid species on apple in California. The injury caused by this aphid, stunting of new growth and production of honeydew, is well known; but a precise economic level of infestation has not been determined. Some growers treat when the aphids become numerous, and others add aphicides to the cover sprays as a precautionary measure.

Winter sprays directed against the overwintering eggs are applied on a regular basis, but this does not prevent summer infestations in California. Garman & Townsend (1952) pointed out that the aphid is "omnivorous"; therefore, destruction of the egg stage on apple does not provide seasonal control.

In California, it has been observed that pyracantha, cotoneaster, quince and hawthorne serve as hosts for overwintering eggs of the apple aphid.

Since summer control of the apple aphid is necessary, a study was undertaken to establish an economic level of infestation so that treatments could be based upon need rather than prevention. At the same time, sampling techniques were studied in order to provide a means of evaluating insecticidal treatments.

It is a well-established observation that the apple aphid prefers the actively growing shoots. Cutright (1930a) stated that the aphids will reproduce only on new and succulent growth. Cutright (1930b) also reported that the growing terminals were four times as attractive to alates, and the aphids produced six times as many young on new leaves. Using this as a basis for sampling, Madsen & Bailey (1959) selected the third leaf down from a terminal

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as a sampling unit when comparing the effects of insecticides against this pest. This provided a means of evaluation, but it was soon evident that a true picture of the population was not obtained. The terminal growth was rapidly reinfested after an insecticide spray unless a systemic compound had been used. The aphids reproduced on the new growth; and as the numbers increased, the aphids began to move downward to older leaves. It was theoretically possible to have essentially the same number of aphids on two plots if the terminal leaf area alone was sampled. In one case the infestation could have spread to the entire shoot while in the other the aphids might be restricted to the terminal growth. Observations indicated that if the aphids infested only the terminal leaves, direct damage to the tree as reflected by honeydew deposits on the fruit was negligible. It was, therefore, necessary to devise a means of sampling which would reflect the numbers and distribution of the aphids on a given shoot. Shands et al. (1954) have pointed out that stratification of a sample was necessary on potatoes to include all the aphid species that might be involved in an infestation. Otake (1958) sampled units on pears in order to determine the trend of the population as the season progressed.

PROCEDURES.—Since the apple aphid first infests the new growth and then spreads downward as populations increase, it seemed desirable to sample three areas on a growing shoot. The terminal, central, and basal portion of a shoot were selected as sampling sites for the 1959 studies. This provided leaf samples consisting of new, mature, and senescent leaves. At each sampling date, 20 leaves were taken per plot from each of the three areas on a shoot. The leaves were placed in cartons, refrigerated, and brushed with the Henderson and McBurnie mite brushing machine. Aphids were then counted with the aid of binocular microscopes.

The field plots were established in a 5-year-old Red Delicious apple orchard near Watsonville, California. Two chemicals were used, the systemic compound dimethoate and the nonsystemic Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate). Each material was applied at monthly and bimonthly intervals to plots consisting of six trees with four replications in a randomized block design. In addition, one plot received a single treatment of dimethoate and was to be retreated when the aphid counts indicated a potentially damaging population.

The materials were applied with a conventional high pressure spray rig and orchard spray guns. Since the trees were small, the gallonage per acre was not calculated. Dimethoate was used at a dosage of 1 pint of 4.0 miscible per 100 gallons, and 25% Diazinon at a rate of 2 pounds wettable per 100 gallons. Aphid samples were taken every 2 weeks throughout the growing season. At harvest, all of the fruit was collected from each tree and examined for the presence of honeydew. Those with honeydew and sooty mold were rated as light (honeydew present but not in amounts that would affect the grade) or heavy (honeydew present in amounts that would downgrade the apples).

The aphid counts are summarized in table 1 and the harvest counts in table 2.

Additional studies were made in 1960 with a slight modification of the sampling technique. Shoots differ in length, and sampling from the three areas described above did not always provide leaves of the same age. To overcome this difficulty, the 3rd, 7th, and 15th leaf from the tip of a shoot were used as sampling units. This provided new, old, and senescent leaves as before but also gave a more uniform sample. Aphid counts were made as previously described.

In the 1960 field plots, dimethoate, Stauffer R-2968 (*O,O*-diethyl dithiophosphorylethyl carbamate), Bayer 30911 (methyl-*O*-methyl *O*-2,4-dichlorophenyl phosphonothioate), and granulated phorate were used as test insecticides. The compounds were applied at the following dosages per 100 gallons: dimethoate, 1 pint 4.0 miscible; Stauffer R-2968, 1 pint 4.0 miscible; and Bayer 30911, 1.5 pints 4.0 miscible. Each compound was applied to four trees with three replications in a randomized block design. As was the case in 1959, the test plot was in a young Red Delicious apple orchard, and a conventional high pressure spray rig was used to apply the treatments. The materials were applied twice during the season, the first spray in June and the second 2 months later. One plot received 10% granulated phorate which was broadcast around the

Table 1.—Apple aphids on Red Delicious apples after treatment with dimethoate and Diazinon. Watsonville, 1959.

MATERIAL	NUMBER OF APHIDS PER 20-LEAF SAMPLE						
	5/28	6/15	6/30	7/13[a]	7/28	8/12	8/27
No. 1—Leaf samples from terminal portion of shoot							
Dimethoate[b]	412	21	2,068	0	132	358	0
Dimethoate[c]	343	57	2,544	147	1,250	360	0
Dimethoate[d]	536	16	2,288	312	1,480	542	1,580
Diazinon[b]	395	350	7,012	0	362	392	72
Diazinon[c]	362	454	8,090	802	3,148	1,830	339
Check	470	3,988	9,141	405	3,028	2,412	1,436
No. 2—Leaf samples from center portion of shoot							
Dimethoate[b]	36	0	17	0	0	0	0
Dimethoate[c]	15	0	10	12	14	12	0
Dimethoate[d]	26	0	15	18	10	12	114
Diazinon[b]	19	0	592	0	10	15	0
Diazinon[c]	22	0	956	146	42	160	0
Check	38	14	1,972	82	162	320	279
No. 3—Leaf samples from basal portion of shoot							
Dimethoate[b]	2	0	0	0	0	0	0
Dimethoate[c]	2	0	0	0	0	0	0
Dimethoate[d]	5	0	6	0	2	6	60
Diazinon[b]	5	0	9	0	0	0	0
Diazinon[c]	2	0	12	20	18	10	0
Check	9	4	60	16	10	20	88

[a] Temperatures: July 9–107° F.; July 10–110° F.
[b] Applied 6/2, 7/7, 8/12.
[c] Applied 6/2, 8/12.
[d] Applied 6/2.

Table 2.—Percentage of apples with honeydew after treatment with dimethoate and Diazinon. Watsonville, 1959.

MATERIAL	FRUIT EXAMINED	PER CENT OF APPLES WITH HONEYDEW[a]	
		Light	Heavy
Dimethoate[b]	440	2.2	0.0
Dimethoate[c]	328	9.4	0.6
Dimethoate[d]	373	16.0	8.3
Diazinon[b]	725	7.5	0.0
Diazinon[c]	454	39.2	10.3
Check	722	32.6	22.5

[a] Harvest date, 9/2.
[b] Applied 6/6, 7/7, 8/12.
[c] Applied 6/6, 8/12.
[d] Applied 6/6.

Table 3.—Apple aphids on Red Delicious apples after treatment with insecticides. Watsonville, 1960.

Material	Aphids per Leaf							
	6/1	6/16	6/24	7/13	7/27	8/10	8/24	9/7
Section 1—third leaf from terminal								
Dimethoate[a]	10.8	0.1	2.9	53.5	155.9	0.0	0.3	0.2
Stauffer[a] R-2968	2.3	0.3	5.3	125.3	174.9	0.2	0.6	8.0
Bayer[a] 30911	8.3	7.1	74.3	202.1	124.3	103.9	69.1	50.3
Phorate[b] granules	9.4	11.5	76.8	140.0	151.1	20.0	23.4	22.7
Check	5.8	21.7	63.2	191.9	142.0	56.4	38.4	39.0
Section 2—seventh leaf from terminal								
Dimethoate[a]	0.03	0.0	0.0	0.6	27.1	0.0	0.0	0.0
Stauffer[a] R-2968	0.1	0.3	0.1	1.6	41.2	0.0	0.1	0.0
Bayer[a] 30911	0.05	0.0	2.3	19.1	18.3	39.9	15.7	22.7
Phorate granules	0.01	0.1	1.6	6.2	25.3	8.2	9.7	16.6
Check	0.1	0.1	1.9	14.7	31.2	9.7	7.6	14.5
Section 3—fifteenth leaf from terminal								
Dimethoate[a]	0.05	0.0	0.0	0.1	12.1	0.0	0.0	0.0
Stauffer[a] R-2968	0.03	0.0	0.0	0.4	6.4	0.0	0.0	0.0
Bayer[a] 30911	0.03	0.0	0.1	6.7	2.3	10.7	12.4	8.8
Phorate[b] granules	0.2	0.0	0.1	1.0	4.1	0.7	1.7	0.0
Check	0.0	0.1	0.7	2.0	5.9	3.7	3.8	13.7

[a] Applied 6/8, 8/3.
[b] Applied 5/4, 7/13.

test trees at the rate of 2 pounds per tree. The first application was made in May to take advantage of the spring rains, and a second treatment was applied in July prior to irrigation.

Aphid samples were taken at 2-week intervals, and the counts are summarized in table 3. At harvest, an evaluation of honeydew-damaged fruit was obtained by the same method as previously described. The data are summarized in table 4.

Results and Discussion.—The data obtained in 1959 were affected by unusually high temperatures which occurred in July and early August in the normally cool coastal apple districts. Temperatures reached 110° F. in early July, and intermittent days when the temperature rose above 95° F. were frequent during July and early August. The hot weather killed large numbers of aphids and was responsible for the drop in population indicated on July 13.

It is evident from the data, however, that when the aphid counts rose to above 100 aphids per 20-leaf sample on the center portion of the shoot, significant honeydew damage was present on the apples at harvest. The trees treated with dimethoate developed high populations on the terminal leaves but not on the leaves sampled from the central and basal portions of the shoot. The plot which received three treatments with Diazinon showed a high count on the center leaves on one count date, but this high population did not result in damaged fruit. The plot that received only two treatments had high populations on the central leaves and aphids on the basal leaves as well. In this case, the fruit was almost as heavily damaged by honeydew as on the check plot. The trees that received a single spray of dimethoate did not show many aphids until August, and this late rise in population did result in damaged fruit. The high temperatures that sharply reduced the aphids in July, however, influenced the plot almost as much as if a treatment had been applied. These data indicate that deleterious amounts of honeydew are produced when the aphids infest the foliage below the terminal growth. An exact number per leaf is probably

Table 4.—Percentage of apples with honeydew after treatment with insecticides. Watsonville, 1960.

Material	Per Cent of Apples with Honeydew[a]		
	Light	Heavy	Total
Dimethoate[b]	0.5	0.0	0.5
Stauffer[b] R-2968	1.3	0.0	1.3
Bayer[b] 30911	19.6	5.8	25.4
Phorate granules[c]	27.5	5.7	33.2
Check	23.6	9.8	33.4

[a] 200 samples per replicate, 600 total; harvest date 9/7.
[b] Applied 6/8, 8/3.
[c] Applied 5/4, 7/13.

not so important as the distribution of the aphids on a growing shoot.

The 1960 data again indicate the value of sampling foliage other than the terminal growth to determine a potentially damaging aphid population. Another evident factor is the length of time aphid numbers remain at a high level. Trees treated with dimethoate showed a steady increase in aphids on the terminal growth following the initial application. On the 7th and 15th leaves, however, high numbers of aphids were present only for a short period prior to the second spray. The same pattern is shown on the trees which received Stauffer R-2968. Neither of these plots had fruit with significant amounts of honeydew. Trees treated with Bayer 30911 and granulated phorate had relatively high numbers of aphids on the lower leaf samples from early July through August. The unsprayed trees showed the same pattern, and all three plots had a high percentage of apples with honeydew. The counts also indicate that when the terminal leaf shows an aphid infestation in the magnitude of 50 or more per leaf, aphids will then be found on the lower leaves.

Conclusions.—The studies over the two seasons indicate that leaf samples from the central portion of growing shoots could serve as a good criterion for determining whether control measures are necessary. If a leaf from this area is infested, the population is potentially damaging. As long as the aphids are restricted to the terminal growth, there will be little if any direct damage to the fruit. It should be possible to avoid multiple treatments for apple aphid control by spraying only when necessary rather than applying an aphicide each time an infestation is present on the terminal leaves of a growing shoot.

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Effect of Insecticides on Emergence of Sugar-Beet Seedlings and on Control of the Sugar-Beet Root Maggot[1]

A. M. Harper,[2] C. E. Lilly,[2] and P. Bergen[3]

Abstract

Both heptachlor-impregnated triple superphosphate (0-43-0) and heptachlor-impregnated ammonium phosphate (11-48-0) at 1 lb. of toxicant per acre significantly reduced damage by the sugar-beet root maggot, *Tetanops myopaeformis* (Röder). Ethion (Niagara Brand Spray Company Limited) at 1 lb. per acre, Trithion® (*O,O*-diethyl *S*(*p*-chlorophenylthio)methyl phosphorodithioate (Stauffer Chemical Company, Portland, Oregon)) at 1 lb. per acre and seed treatments with heptachlor at both 1 and 2 oz. per acre also reduced maggot damage. The heptachlor-impregnated ammonium phosphate at 1 lb. of toxicant per acre, a seed treatment with heptachlor at 4 oz. per acre, and Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate (Chemagro Corporation, Kansas City, Mo.)) at 1 lb. per acre significantly reduced emergence of seedlings, and the latter treatment gave little control of the maggot. The sugar-beet root maggot was most effectively controlled in southern Alberta with 1 lb. of heptachlor per acre placed in the drill row with the seed or 3 lb. per acre of heptachlor placed in a 5-inch band over the row. These two treatments, however, left residues of heptachlor and heptachlor epoxide in the sugar-beet roots at harvest.

The sugar-beet root maggot, *Tetanops myopaeformis* (Röder), is a pest of sugar beets in southern Alberta as well as in the following areas of western North America: North Dakota (Callenbach et al. 1957), Idaho (Jones et al. 1952), and Manitoba (Allen et al. 1959).

Heptachlor-impregnated ammonium phosphate fertilizer (11–48–0) placed in the drill row at time of seeding and other heptachlor formulations have given control of the sugar-beet root maggot (Lilly et al. 1961). No other insecticide tested in Alberta gave control of the maggot equal to that obtained with heptachlor (Harper et al. 1961).

In this paper three seed treatments with heptachlor and three new organophosphate treatments are compared with the recommended heptachlor-impregnated ammonium phosphate treatment. Results are also given on the effect of heptachlor, heptachlor-impregnated fertilizer, and nontreated fertilizer on germination of beets and on control of the maggot. Information is also given on residues of heptachlor present on foliage and roots of sugar beets.

Materials and Methods.—This experiment was conducted on irrigated land at two locations near Taber, Alberta. One field was 1 mile southeast and the other approximately 8 miles northeast of Taber in an area called Big Bend. The field at Taber had been planted to sugar beets the previous year and the one at Big Bend to potatoes. Both farms had been infested with sugar-beet root maggots in 1959. The soil at Taber was 75% Cavendish fine sandy loam and 25% Chin loam with a pH of 8.1 and at Big Bend 75% Cavendish fine sandy loam and 25% Hemaruka sandy loam with a pH of 7.4. Prior to seeding, the field at Taber had been fertilized by the farmer with 50 lb. of ammonium phosphate (11–48–0) and 100 lb. of ammonium nitrate (33.5–0–0) in a broadcast and plow application. The Big Bend field had been fertilized with 80 lb. of ammonium phosphate and 160 lb. of ammonium nitrate in a broadcast and harrow application. At the time of seeding, the soil had a moisture content, based on oven-dry weight, of 16.8% at Taber and 13.9% at Big Bend. Between May 13 and May 25, 1.11 inches of rain fell on these fields.

The experiment was set out in a randomized block design with plots 60 ft. long and four drill rows wide. The drill rows were 22 inches apart. The following 14 treatments were replicated five times at each location:

Treatment	Rate of actual toxicant acre
1. Check	—
2. Fertilizer only (11-48-0 at 100 lb./acre)	—
3. Fertilizer (11-48-0 at 100 lb./acre)+heptachlor	1 lb.
4. Fertilizer only (0-43-0 at 100 lb./acre)	—
5. Fertilizer (0-43-0 at 100 lb./acre)+heptachlor	1 lb.
6. Band of granulated heptachlor over the drill row	3 lb.
7. Granulated heptachlor in the row	1 lb.
8. Seed coated with methyl cellulose (methocel) at 15 ml. 1% methyl cellulose solution per 100 g. seed	—
9. Seed coated with methyl cellulose and heptachlor	1 oz.
10. Seed coated with methyl cellulose and heptachlor	2 oz.
11. Seed coated with methyl cellulose and heptachlor	4 oz.
12. Granulated Trithion® (*O,O*-diethyl S(*p*-chlorophenylthio)methyl phosphorodithioate (Stauffer Chemical Company, Portland, Oregon)) in the row	1 lb.
13. Granulated ethion (Niagara Brand Spray Company Limited) in the row	1 lb.
14. Granulated Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate (Chemagro Corporation, Kansas City, Mo.)) in the row	1 lb.

All seed was planted at the rate of 5.36 lb. of monogerm seed per acre with a shoe drill that placed the fertilizer and insecticides in the drill row. Attached to the seed drill were "V" belts that metered the insecticides and fertilizers into the row with much greater precision than could be obtained with commercial drills. Plots were seeded and harvested at Taber on May 11 and September 19 and at Big Bend on May 16 and September 16. Beets were thinned to an optimum of 120 beets per 100 ft. of row during the week of June 13 to 20.

The stands before thinning were determined on June 8

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[2] Entomologists, Research Station, Lethbridge.

[3] Plant Breeder, Canadian Sugar Factories Limited, Taber, Alberta.

and 9 by counting (1) the number of beets in 50 ft. of each of the two central rows of each plot, (2) the number of beets in 100 in. of each of the the two central rows, and (3) the number of "beet-containing" inches in 100 in. of each of the two central rows. The stands after thinning were determined on June 23 and 24 by counting (1) the "beet-containing" feet in 50 ft. of each of the two central rows and (2) the number of beets in 50 ft. of each of the same two rows. Data on the number of "beet-containing" inches or "beet-containing" feet indicate the uniformity of distribution of the beets in the row. Summer stands were determined, on July 28 at Taber and on August 2 at Big Bend, by counting the number of beets in 50 ft. of each of the two central rows of each plot. The harvest stands, also taken on 50 ft. of each of the two central rows, are not comparable directly to the stands after thinning or to the summer stands because the former do not include beets of less than 1 in. in diameter; these were not counted as they would not normally be harvested with mechanical harvesters.

At harvest the beets were rated for maggot damage, the ratings ranging from 0 for undamaged beets to 3 for severely damaged. Because of surface scarring not attributable to insect feeding, it was not possible to accurately rate the beets for damage in the first two replicates at Big Bend. This scarring was similar to that caused by boron deficiency. The mean damage ratings are based on only three replicates at that location and five at Taber.

All beets more than 1 in. in diameter from the two central rows of each plot were washed, weighed, and sampled with a multi-saw rasp for sugar determination. Sugar per acre was determined by multiplying beet yield by percentage sugar. Just prior to harvest, beets were taken from heptachlor-treated plots for analysis of residue.

All data were compared at the 5% level of significance by Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION.—*Stand Before Thinning.*—When the stands before thinning were determined on the basis of "beet-containing" inches (table 1), those of the check and of the Trithion-, ethion-, and methocel-seed-treated plots were not significantly different from each other at either location, but all were significantly higher than those in the plots in which the seed had been treated with 4 oz. of heptachlor or in those plots treated with Bayer 29493 or heptachlor-impregnated ammonium phosphate fertilizer. There were no significant differences in stand at either location between those plots treated with 1 lb. of heptachlor placed in the drill row and those treated with 3 lb. of heptachlor in a band over the row. Neither was significantly different in stand from the check plots or those with methyl-cellulose-coated seed. The plots treated with triple superphosphate fertilizer did not have significantly lower stands at either location; however, the plots treated with heptachlor-impregnated triple superphosphate had stands that were significantly lower

Table 1.—Mean stands of sugar beets before thinning in plots treated with various insecticides for control of the sugar-beet root maggot, Taber and Big Bend, Alberta, 1960.

TREATMENT	NO. OF "BEET-CONTAINING" INCHES[a]	TREATMENT	NO. OF BEETS IN 200 INCHES	TREATMENT	NO. OF BEETS IN 100 FEET
		Taber			
Ethion	54[b]	Check	68	Check	431
Check	53	Ethion	67	Trithion	373
Methocel	51	Methocel	63	Methocel	367
Trithion	50	Trithion	60	Ethion	360
H[c] 1 lb.	45	H 1 lb.	58	H 1 lb.	328
0-43-0	44	0-43-0	56	Seed 2 oz. H	305
Seed 1 oz. H	44	Seed 2 oz. H	55	Band 3 lb. H	299
Seed 2 oz. H	43	Seed 1 oz. H	54	0-43-0	293
Band 3 lb. H	39	Band 3 lb. H	49	Seed 1 oz. H	291
0-43-0+H	39	0-43-0+H	48	0-43-0+H	269
11-48-0	31	11-48-0	40	11-48-0	245
Seed 4 oz. H	30	Seed 4 oz. H	36	Seed 4 oz. H	224
Bayer 29493	25	Bayer 29493	28	11-48-0+H	140
11-48-0+H	20	11-48-0+H	25	Bayer 29493	135
		Big Bend			
Ethion	58	Ethion	75	Ethion	418
Check	53	Check	67	Check	390
Methocel	52	H 1 lb.	65	Trithion	385
0-43-0	52	Trithion	63	0-43-0	380
H 1 lb.	50	0-43-0	63	H 1 lb.	367
Trithion	48	Methocel	61	Methocel	363
Seed 1 oz. H	42	Seed 1 oz. H	55	Band 3 lb. H	309
Band 3 lb. H	41	Band 3 lb. H	54	Seed 2 oz. H	286
Seed 2 oz. H	38	0-43-0+H	50	Seed 1 oz. H	275
0-43-0+H	38	11-48-0	46	0-43-0+H	234
11-48-0	38	Seed 2 oz. H	46	11-48-0	228
Seed 4 oz. H	34	Seed 4 oz. H	42	Seed 4 oz. H	215
Bayer 29493	29	Bayer 29493	37	Bayer 29493	190
11-48-0+H	22	11-48-0+H	26	11-48-0+H	161

[a] "Beet-containing" inches in 200 inches.
[b] Means connected by the same vertical line are not significantly different at P = .05.
[c] H = heptachlor.

Table 2.—Mean stands of sugar beets after thinning in plots treated with various insecticides for control of the sugar-beet root maggot, Taber and Big Bend, Alberta, 1960.

Initial Stand After Thinning (100 row-feet)				Summer Stand (100-row-feet)		Harvest Stand (100 row-feet)	
Treatment	No. of "Beet-Containing" Feet	Treatment	No. of Beets	Treatment	No. of Beets	Treatment	No. of Beets
			Taber				
Check	94 [a]	Check	126	Trithion	120	Trithion	114
Methocel	94	Trithion	123	Seed 1 oz. H	117	Seed 1 oz. H	114
Seed 1 oz. H[b]	92	Methocel	122	Seed 2 oz. H	116	Seed 2 oz. H	111
Trithion	91	Seed 1 oz. H	121	Check	116	Band 3 lb. H	110
Seed 2 oz. H	91	Ethion	120	Methocel	114	Ethion	110
Ethion	90	Seed 2 oz. H	117	Ethion	114	H 1 lb.	109
Band 3 lb. H	88	H 1 lb.	116	H 1 lb.	112	Methocel	108
H 1 lb.	88	Band 3 lb. H	115	Band 3 lb. H	112	0-43-0+H	106
0-43-0+H	86	0-43-0+H	112	0-43-0+H	109	Check	105
0-43-0	85	0-43-0	111	Seed 4 oz. H	106	Seed 4 oz. H	100
Seed 4 oz. H	85	11-48-0	109	0-43-0	102	0-43-0	95
11-48-0	81	Seed 4 oz. H	108	11-48-0	95	11-48-0	79
Bayer 29493	66	11-48-0+H	82	11-48-0+H	81	11-48-0+H	75
11-48-0+H	62	Bayer 29493	81	Bayer 29493	78	Bayer 29493	75
			Big Bend				
Methocel	92	Methocel	119	Methocel	113	Trithion	107
Trithion	91	0-43-0	116	Ethion	111	Ethion	107
Ethion	90	Ethion	116	Trithion	109	Methocel	102
0-43-0	89	Check	115	0-43-0	108	Seed 1 oz. H	101
Check	89	Trithion	115	Seed 1 oz. H	108	Check	101
H 1 lb.	88	H 1 lb.	113	H 1 lb.	107	H 1 lb.	101
Seed 1 oz. H	85	Seed 1 oz. H	113	Check	105	0-43-0	100
Seed 2 oz. H	82	Band 3 lb. H	106	Band 3 lb. H	102	Band 3 lb. H	100
Band 3 lb. H	82	11-48-0	103	Seed 2 oz. H	99	Seed 4 oz. H	96
11-48-0	80	Seed 2 oz. H	102	Seed 4 oz. H	97	Seed 2 oz. H	92
Seed 4 oz. H	78	Seed 4 oz. H	102	11-48-0	90	11-48-0	84
0-43-0+H	68	0-43-0+H	90	0-43-0+H	83	0-43-0+H	76
Bayer 29493	63	Bayer 29493	79	Bayer 29493	74	Bayer 29493	72
11-48-0+H	62	11-48-0+H	77	11-48-0+H	72	11-48-0+H	66

[a] Means connected by the same vertical line are not significantly different at $P = .05$.
[b] H = heptachlor.

than those of the check. At both locations plots treated with ammonium phosphate fertilizer or heptachlor-impregnated ammonium phosphate were significantly lower in stand than the check plots.

Stands before thinning determined by the other two methods of sampling showed a similar relationship between the treatments and the checks. It appeared, however, that counting the number of beets in 100 ft. of row in each plot gave greater distinction between treatments than counting the number in 200 in. (table 1).

Stand After Thinning.—At Taber, plots treated with Bayer 29493 and those treated with heptachlor-impregnated ammonium phosphate fertilizer had stands after thinning that were significantly lower than in all other plots (table 2).

At Big Bend the plots treated with Bayer 29493 and those treated with heptachlor-impregnated ammonium phosphate fertilizer had stands after thinning that were significantly lower than the check and all other treatments except the heptachlor-impregnated triple superphosphate (table 2).

As shown in table 2, the two methods of counting gave comparable estimates of the relative reduction in stands caused by the various treatments. The counts also showed that the plants in all plots were spaced more or less uniformly in the row.

Summer and Harvest Stands.—At Taber, plots treated with Bayer 29493 or heptachlor-impregnated ammonium phosphate had significantly lower stands during the summer than all other plots, and at harvest lower stands than all plots except those treated with ammonium phosphate fertilizer. At Big Bend the same two treatments had stands lower than all other treatments except the ammonium phosphate fertilizer and the heptachlor-impregnated triple superphosphate (table 2).

Damage Rating.—At Taber, beets from all plots without insecticide treatment, *i.e.*, the check, the methyl-cellulose-coated seed, and the fertilizer treatments of ammonium phosphate and triple superphosphate, had significantly more magogt damage than those from all other plots (table 3). The beets from plots treated with 1 lb. of heptachlor had significantly less damage than those from all other plots except the ones treated with 3 lb. of heptachlor, heptachlor-impregnated ammonium phosphate, and heptachlor-impregnated triple superphosphate. Beets from plots treated with the latter three chemicals had significantly less damage than those from all other treated plots except the 4-oz. seed treatment.

At Big Bend, beets from plots with ammonium phosphate fertilizer, triple superphosphate fertilizer, and methyl-cellulose-coated seed had significantly more damage than beets from all other treated plots (table 3). Beets from the check plots had significantly more damage than those grown from the heptachlor-treated seed or in

Table 3.—Damage by the sugar-beet root maggot in plots treated with various insecticides, Taber and Big Bend, Alberta, 1960.

Treatment	Damage Rating	Treatment	Percentage of Beets Infested
	Taber		
11-48-0	2.12 [a]	Methocel	96
Methocel	2.08	11-48-0	96
Check	2.07	Check	96
0-43-0	2.04	0-43-0	95
Bayer 29493	1.72	Bayer 29493	88
Ethion	1.11	Ethion	70
Seed 1 oz. H [b]	0.99	Seed 1 oz. H	69
Trithion	0.94	Trithion	60
Seed 2 oz. H	0.86	Seed 2 oz. H	56
Seed 4 oz. H	0.65	Seed 4 oz. H	47
0-43-0+H	0.45	0-43-0+H	34
Band 3 lb. H	0.39	11-48-0+H	28
11-48-0+H	0.38	H 1 lb.	25
H 1 lb.	0.29	Band 3 lb. H	24
	Big Bend		
11-48-0	1.56	11-48-0	88
Methocel	1.38	Methocel	77
0-43-0	1.29	0-43-0	75
Check	0.78	Check	52
Bayer 29493	0.66	Bayer 29493	50
Ethion	0.43	Ethion	35
Seed 1 oz. H	0.30	Seed 1 oz. H	27
Seed 2 oz. H	0.29	Seed 4 oz. H	24
11-48-0+H	0.27	Seed 2 oz. H	22
Seed 4 oz. H	0.25	11-48-0+H	22
0-43-0+H	0.23	0-43-0+H	21
H 1 lb.	0.17	H 1 lb.	16
Trithion	0.16	Trithion	14
Band 3 lb. H	0.14	Band 3 lb. H	13

[a] Means connected by the same vertical line are not significantly different at P = .05.
[b] H = heptachlor.

the plots treated with heptachlor or Trithion. There was no significant difference in damage between plots with the heptachlor treatments or between those and the ones treated with Trithion or ethion.

Percentage of Beets Exhibiting Damage.—At Taber, those plots not treated with insecticide and those treated with Bayer 29493 were not significantly different from each other in percentage damage but they all had a significantly higher damage than other plots (table 3). The plots treated with heptachlor-impregnated ammonium phosphate and with 1 or 3 lb. of heptachlor had significantly less damage than all other plots except those treated with heptachlor-impregnated triple superphosphate. The plots with the 4-oz.-heptachlor seed treatment had significantly less damage than the ones with the 1-oz. treatment, and neither was significantly different from the ones with the 2-oz. treatment.

At Big Bend, the plots with methyl-cellulose-treated seed and those with ammonium phosphate and triple superphosphate fertilizer had significantly more damage than all others (table 3). The check plots had more damage than those with heptachlor-treated seed or those treated with heptachlor or Trithion. All the latter plots were not significantly different from each other in percentage damage.

Yield of Beets.—At Taber, the plots treated with 3 lb. of heptachlor produced yields of beets significantly higher than those produced in all other plots (table 4). The plots treated with 1 lb. of heptachlor had yields significantly higher than all plots not treated with insecticide and those treated with heptachlor-impregnated ammonium phosphate or Bayer 29493. The latter two treatments were phytotoxic, resulting in significantly lowered stands, which may account for the low yields. There was no significant difference in yields of beets in plots that had been sown with seed treated with any of the three rates of heptachlor. The plots sown with seed treated with 2 or 4 oz. of heptachlor and those treated with ethion, Trithion, and heptachlor-impregnated triple superphosphate had significantly higher yields of beets than the check.

At Big Bend there were no significant differences in yields (table 4). However, the plots treated with 3 lb. of heptachlor gave the highest yield, followed by plots treated with ethion, 1 lb. of heptachlor, and the seed treatments.

Yield of Sugar.—At Taber the yield of sugar from beets in plots treated with 3 lb. of heptachlor per acre was not significantly different from the yield in plots treated with 1 lb. of heptachlor per acre, but it was significantly higher than in all other plots (table 4). In plots treated with 1 lb. per acre of heptachlor, ethion, Trithion, and heptachlor-impregnated triple superphosphate, and in plots where heptachlor-treated seed was used, the yields were significantly higher than in the check plots and in those treated with Bayer 29493, ammonium phosphate fertilizer, and triple superphosphate fertilizer or seeded with methyl-cellulose-coated seed.

At Big Bend there were no significant differences in yields of sugar but the plots treated with 3 lb. per acre of heptachlor again gave the highest yield, followed by plots treated with ethion, those sown to seed treated with 2 oz. of heptachlor, and those treated with 1 lb. per acre of heptachlor (table 4).

Percentage Sugar.—At Taber there were no significant differences in percentage sugar. At Big Bend, the percentage sugar was significantly higher in the plots sown with seed treated with 2 oz. of heptachlor and in the plots treated with Trithion than in the check plots and in those treated with ammonium phosphate and triple superphosphate.

Residues of Heptachlor.—Table 5 gives information on heptachlor residues in the sugar-beet leaves and roots. The residues were determined by personnel of the Velsicol Chemical Corporation, Chicago, Illinois, who indicated that the sensitivity of the quantitative test falls within the range 0.03 and 0.08 p.p.m. The qualitative test, which takes the information from the spectrogram, is more indicative of the true evidence of heptachlor and heptachlor epoxide. Only the 1 lb. of granulated heptachlor in the drill row and the 3 lb. over the row left residues in the beet root at harvest. None of the treatments left residues in the leaves.

CONCLUSIONS.—Both heptachlor-impregnated triple superphosphate fertilizer and heptachlor-impregnated ammonium phosphate fertilizer controlled the sugar-beet root maggot but significantly reduced the stands before thinning. McDonald (1960) also found that ammonium phosphate fertilizer impregnated with solvents and heptachlor-solvent combinations, placed in contact with germinating sugar-beet seeds, was phytotoxic. In this study the ammonium phosphate fertilizer alone caused a significant reduction in the stand of beets before thinning. The stand was more severely reduced where heptachlor

Table 4.—Yields of beets and sugar from plots treated with various insecticides for control of the sugar-beet root maggot, Taber and Big Bend, Alberta, 1960.

TREATMENT	BEETS (TONS/ACRE)	TREATMENT	PERCENTAGE SUGAR	TREATMENT	SUGAR (LB./ACRE)
		Taber			
Band 3 lb. H[a]	15.74[b]	Seed 1 oz. H	17.3	Band 3 lb. H	5,228
H. 1 lb.	14.56	Ethion	17.1	H. 1 lb.	4,853
Seed 2 oz. H	14.04	Trithion	16.9	Seed 2 oz. H	4,736
Ethion	13.88	0-43-0+H	16.8	Ethion	4,725
Seed 4 oz. H	13.72	11-48-0+H	16.8	Trithion	4,581
Trithion	13.59	11-48-0	16.7	Seed 1 oz. H	4,581
0-43-0+H	13.55	H. 1 lb.	16.7	0-43-0+H	4,536
Seed 1 oz. H	13.26	Seed 2 oz. H	16.7	Seed 4 oz. H	4,525
11-48-0+H	13.05	Methocel	16.6	11-48-0+H	4,383
Check	12.15	Band 3 lb. H	16.6	Bayer 29493	3,980
Bayer 29493	12.13	Seed 4 oz. H	16.5	0-43-0	3,929
0-43-0	11.88	0-43-0	16.5	Check	3,917
Methocel	11.64	Bayer 29493	16.4	Methocel	3,862
11-48-0	10.75	Check	16.1	11-48-0	3,598
		Big Bend			
Band 3 lb. H	10.54	Seed 2 oz. H	15.9	Band 3 lb. H	3,280
Ethion	10.48	Trithion	15.9	Ethion	3,170
H. 1 lb.	10.04	Seed 1 oz. H	15.6	Seed 2 oz. H	3,089
Seed 4 oz. H	9.70	0-43-0+H	15.6	H 1 lb.	3,076
Seed 2 oz. H	9.68	Band 3 lb. H	15.6	Trithion	3,031
Seed 1 oz. H	9.60	Methocel	15.5	Seed 1 oz. H	3,010
Trithion	9.54	Bayer 29493	15.5	Seed 4 oz. H	2,994
11-48-0	9.53	Seed 4 oz. H	15.4	Methocel	2,897
Check	9.46	H 1 lb.	15.3	11-48-0	2,862
0-43-0	9.44	11-48-0+H	15.3	0-43-0	2,841
Methocel	9.33	Ethion	15.3	Bayer 29493	2,840
Bayer 29493	9.19	0-43-0	15.1	0-43-0+H	2,835
0-43-0+H	9.16	Check	15.0	Check	2,832
11-48-0+H	9.10	11-48-0	15.0	11-48-0+H	2,826

[a] H = heptachlor.

[b] Means connected by the same vertical line are not significantly different at P = .05.

was impregnated in the fertilizer. However, where heptachlor alone was applied at 1 lb. per acre the stand was not significantly lower than in the check. The reduction in stand caused by the fertilizer or the heptachlor-impregnated fertilizer is not serious with present seeding rates but will become more important as monogerm seed is used and as beets are seeded at low rates with precision planters.

Seed treated with 1 to 4 oz. of heptachlor gave control of the maggot only slightly lower than that obtained with the application of granules in the row. Four ounces of heptachlor applied to the seed with methyl cellulose reduced stands before thinning and did not give a significant increase in control of the maggot over the 2-oz. application. There was no significant difference in yield between the three levels of heptachlor applied. These results indicate that seed treatments could be used as an alternative to heptachlor-impregnated fertilizer in southern Alberta. Because the fungicide captan also has to be applied to the seed, there may be some mechanical difficulties involved in applying the heptachlor and the fungicide to the seed on a commercial basis.

Table 5.—Residues of heptachlor and heptachlor epoxide in sugar beets from plots treated to control the sugar-beet root maggot, Taber, Alberta, 1960.

PART ANALYZED	HEPTACHLOR TREATMENT	HEPTACHLOR P.P.M.	HEPTACHLOR Quality Test[a]	HEPTACHLOR EPOXIDE P.P.M.	HEPTACHLOR EPOXIDE Quality Test[a]	Total P.P.M.
Foliage	Check	0.04	No	0.04	No	0.08
Foliage	4-oz./5.36 lb. seed	.03	No	.05	No	.08
Foliage	1 lb./acre in fertilizer	.01	No	.04	No	.05
Foliage	1 lb./acre, granules	.01	No	.06	No	.07
Foliage	3 lb./acre granules	.03	No	.05	No	.08
Root	Check	.02	No	.03	No	.05
Root	4-oz./5.36 lb. seed	.02	No	.03	No	.05
Root	1 lb./acre in fertilizer	.01	No	.04	No	.05
Root	1 lb./acre, granules	.16	Yes	.10	Yes	.26
Root	3 lb./acre, granules	.18	Yes	.30	Yes	.48

[a] Qualitative test indicates whether or not critical qualitative absorption band is detected in the spectrogram.

The tests conducted in 1960 and in 1956 and 1959 indicate that heptachlor is the most effective insecticide for control of the sugar-beet root maggot in southern Alberta.

When granulated heptachlor is applied at 1 lb. per acre in the drill row or at 3 lb. per acre in a band over the row, good maggot control is obtained and stands are not reduced but residues of heptachlor and heptachlor epoxide remain in the roots at harvest. Because beet pulp is fed to livestock these methods of applying heptachlor cannot be recommended in Alberta with the present tolerance limits for residues of heptachlor and heptachlor epoxide.

Bayer 29493 reduced seedling stands and gave little control of the maggot. Both ethion and Trithion reduced maggot damage and were not phytotoxic to beets when applied in the drill row in a granulated formulation at 1 lb. per acre. If the maggot develops resistance to heptachlor it may be possible to use Guthion (Harper et al. 1961), ethion, or Trithion which have shown promise.

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Effectiveness of Malathion Dust as a Protectant for Farm-Stored Corn in Louisiana[1]

E. H. FLOYD, *Louisiana Agricultural Experiment Station, Baton Rouge*

ABSTRACT

Results are given of studies conducted to evaluate the protective values of malathion and pyrethrum grain protectants against damage to corn in Louisiana caused by stored grain insects, especially the rice weevil, *Sitophilus oryzae* (L.). Both shelled and snapped corn were used in the studies. Malathion gave some protection to stored corn from progressive insect damage during storage; however, satisfactory protection was not obtained at any dosage level tested. Malathion was more effective than pyrethrum. Greater protection was obtained in shelled corn than was possible in snapped corn.

Failure in Louisiana to protect corn in storage from insect damage with malathion or pyrethrum is very likely due to the high initial infestation incurred in the field and favorable conditions of temperature, relative humidity, and grain moisture for insect development during much of the storage period.

Several species of stored-grain insects damage corn in Louisiana. The rice weevil, *Sitophilus oryzae* (L.) (Floyd & Newsom 1959) is responsible for most of the damage. Damage by the Angoumois grain moth, *Sitotroga cerealella* (Olivier), increases with time in storage. An average of 10% of the kernels was found to be damaged by harvest time in Louisiana (Floyd et al. 1959). Kernel damage increased to 31% after approximately 9 months' storage. The rice weevil was responsible for practically all the early damage. The Angoumois grain moth was responsible for 17% of the total damage after 9 months with the remainder being caused by the rice weevil.

Research has been in progress several years at the Louisiana Agricultural Experiment Station to find a suitable protectant for alleviating insect damage to corn in storage. The efficacy of many materials has been investigated in laboratory tests and under farm storage conditions. The pyrethrum and pyrethrum-compounds containing synergists provided very little protective value to corn stored in Louisiana. Lindane, adsorbed on sawdust and scattered among the snapped ears or in the shelled grain at storage time at dosages as low as 2 p.p.m. gave complete protection of the grain from rice weevil damage throughout the storage period. (Floyd & Smith 1953, Floyd & Newsom 1956). In order to fully protect shelled corn from damage by the Angoumois grain moth a dosage of 10 p.p.m. lindane was required. Since lindane is adsorbed by the grain and is recoverable by chemical analyses in the amount of approximately 1 p.p.m. regardless of original dosage applied, it cannot be recommended for use on grain.

The high level of rice weevil infestation which commonly develops in corn before it is harvested in Louisiana, along with conditions favorable for development of the insect during much of the storage season, probably accounts for the failure of mild protectants such as pyrethrum to effect adequate protection of corn.

The application of malathion for control of stored-grain insects has been investigated by several workers. Lindgren et al. (1954) reported on the effectiveness of malathion dust and spray for the control of several insect pests of wheat. The rice weevil was found to be very susceptible to the chemical. Kill was obtained 7 months after treatment at dosages of 8 and 16 p.p.m. Dust formulations were superior to liquid formulations. Grain moisture was considered to have a probable influence on the effectiveness of malathion as a grain protectant.

Vincent & Lindgren (1957) showed that malathion in laboratory tests was effective against several species of stored-grain insects. Womack & LaHue (1959) reported on the effectiveness of malathion spray as a protectant for shelled corn. Rice weevil control and maintenance of test weight of the grain were closely associated with dosage rates. A dosage of 20 p.p.m. was found necessary to give adequate protection for 8 months' storage.

Gunther et al. (1958) determined that the half-life value of malathion on wheat at 8 p.p.m. was 5 months for *S. oryzae*. Strong & Sbur (1960) reported that the effectiveness of malathion against three species of stored-grain insects was reduced with increases in the grain moisture content. Fourteen per cent appeared to be the critical level for persistence of biologically effective malathion residue on wheat.

This paper is concerned with the evaluation of malathion and pyrethrum as grain protectants for stored

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corn in Louisiana during the 1958–1959 and 1959–1960 storage seasons.

PROCEDURE.—*Experiment No. 1.—Snapped corn test—1958–1959.*—On October 6, 1958 an experiment was set up to evaluate the effectiveness of several dosage levels of malathion and synergized pyrethrum (Pyrenone) grain protectant dusts for control of the rice weevil in snapped corn.

Funk's 740, a popular yellow hybrid, was used. The grain moisture at time of storage was 15.5% and the grain weight averaged 57.3 pounds per bushel.

Each treatment was replicated three times with each replicate consisting of 2 bushels (140 pounds) of field-run ears. The experimental bin was a 55-gallon steel drum. The 2 bushels approximately filled the drum. The tops of the drums were left open but the corn was protected from rodents with a $\frac{1}{4}$-inch mesh hardware cloth cover.

The harvested corn was piled in the center of a warehouse floor. Ears were picked at random from all sections of the pile, placed in a bushel basket then transferred to the experimental bin. As each bin was being filled, the appropriate amount of insecticide was added with each 6-inch rise. In all cases the top surface of the corn in each bin received some insecticide.

The initial infestation by the rice weevil was determined as follows: From each basket of corn, five ears were removed at random. After all the bins were filled these selected ears were shucked and the percentage of infested ears was determined. Then the ears were shelled, the grain thoroughly mixed and the number of kernels damaged or infested in 10 lots of 100 each was determined. Egg punctures were not considered in this study. Thirty-two per cent of the ears and 7% of the kernels were found to be infested by the rice weevil at time of storage.

The experimental bins were stored inside a ventilated metal warehouse used for storage of farm equipment and shelter for farm animals.

Treatments were as follows:

1. Malathion grain protectant dust (1% malathion) at the rate of 60 pounds per 1,000 bushels (10.5 p. p. m.).
2. Same used at 180 pounds per 1,000 bushels (31.5 p. p. m.).
3. Same used at 360 pounds per 1,000 bushels (63 p. p. m.).
4. Pyrenone grain protectant—100 pounds per 1,000 bushels (0.9 p. p. m. pyrethrins; 14 p. p. m. piperonyl butoxide).
5. Control.

Examinations were made after 1, 2, 3, 8 and 10 months to determine rice weevil damage, the grain moisture and the grain test weight per bushel. Twenty ears selected at random were removed from each replicate at each examination. The ears were removed with as little disturbance of the bulk of corn as was possible.

Experiment No. 2.—Shelled corn test—1958–1959.—Shelled Shoepeg corn of 16.5% moisture content and 53.2 pounds per bushel at time of storage was used in this study. The corn was grown in the summer of 1958. The test was begun September 25, 1958. At time of storage $1\frac{1}{2}$% of the kernels were damaged by the rice weevil. Single replicates consisted of 28 pounds of corn (approximately $\frac{1}{2}$ bushel) stored in plywood boxes which measured 12″×12″×12″ inside. The top was open but the corn was protected from rodents by a $\frac{1}{4}$-inch mesh hardware cloth cover.

The entire lot of shelled corn was first placed in a single heap, turned several times with shovels then placed in the experimental bins. As each box was being loaded the appropriate amount of insecticide was added in the stream of grain as it entered the box. After the boxes were filled they were equipped with the hardware cloth covers and arranged randomly in the warehouse for storage. Treatments were replicated three times.

The initial kernel damage was determined by removing 10 sub-samples of 100 kernels each taken at several locations from the large pile of mixed corn and examining the kernels under a magnifying lens for infestation and/or damage by stored-grain insects. The grain moisture and the weight per bushel were determined immediately before the storage boxes were filled.

The variety of corn used in this test, Shoepeg, is very susceptible to infestation by the rice weevil.

Treatments were as follows:

1. Malathion grain protectant dust (1% malathion) at the rate of 60 pounds per 1,000 bushels (11.3 p. p. m.).
2. Same used at rate of 180 pounds per 1,000 bushels (33.9 p. p. m.).
3. Pyrenone grain protectant—100 pounds per 1,000 bushels. (0.9 p. p. m. pyrethrins; 14 p. p. m. piperonyl butoxide).
4. Check.

Approximately 150-gram samples were taken from the top to the bottom of the grain from each replicate after 1, 2, 4, 6, 9 and 11 months. These samples were used to ascertain the degree of damage caused by stored-grain insects at different intervals following treatment.

Experiment No. 3.—Snapped corn test—1959–1960.—This test was initiated on October 15, 1959 using 1% malathion dust at several dosage levels on snapped Louisiana 521 corn that contained a very low level of rice weevil infestation at time of storage. This low level of infestation was obtained by the removal of all ears at harvest that showed evidence of bird damage. Twelve per cent of the ears showed infestation by the rice weevil. The mean kernel damage caused by the rice weevil was 0.2%. At the time of storage the grain moisture was 15.8% and the test weight per bushel was 56.8 pounds.

Treatments were replicated four times. Each replicate consisted of 1 bushel of snapped corn (70 pounds) stored in a 25-gallon metal can. The tops were left open, but the ears were protected from rodent damage by a hardware cloth cover. The experimental bins were stored inside an unheated, ventilated metal warehouse.

Treatments were as follows:

1. One per cent malathion grain dust—60 pounds per 1,000 bushels (10.5 p. p. m.).
2. Same—80 pounds per 1,000 bushels (14 p. p. m.).
3. Same—120 pounds per 1,000 bushels (21 p. p. m.).
4. Same—240 pounds per 1,000 bushels (42 p. p. m.).
5. Malathion emulsifiable concentrate absorbed on sawdust and used at 16 p. p. m.
6. Same—at 32 p. p. m.
7. Control.

Treatments were applied in a manner similar to that described in experiment 1. Applications were made September 15, 1959. The experiment was terminated August 20, 1960.

Records of rice weevil damage were made after 6, 9 and

Table 1.—Protective value of malathion and pyrethrum applied to snapped corn against damage by the rice weevil in storage[a] October, 1958–August, 1959.

Dust Treatment (lb./1,000 bu.)	Per Cent of Ears Visibly Infested After Months Shown					Per Cent of Kernels Damaged After Months Shown					Grain Moisture (%) After		Grain (lb./bu.) After	
	1	2	3	8	10	1	2	3	8	10	1 Month	10 Months	1 Month	10 Months
1% malathion, 60	83	87	93	98	100	27	18	35	35	53	13.1	14.4	56.0	48.3
1% malathion, 180	83	77	93	88	97	24	21	34	22	28	12.9	13.4	55.6	53.6
1% malathion, 360	90	83	90	97	95	15	19	27	25	30	13.2	13.9	57.2	53.9
Pyrenone grain protectant, 100	87	90	97	95	98	14	14	30	41	60	13.0	13.9	58.9	49.4
Control	90	93	100	100	100	24	27	45	48	72	13.3	14.4	55.8	41.3
L.S.D. at 1% level							13		17	22				

[a] At time of storage 32% of the ears and 7% of the kernels were infested by rice weevils.

11 months' storage. Records for the first two examinations were made by removing 20 ears at random from each replicate. These ears were shucked and the percentage infested by the rice weevil was determined. The ears were then shelled, the grain mixed and the percentage of kernels damaged by the rice weevil was determined. The grain moisture and test weight per bushel were determined for each sample at each examination. At the final examination all the remaining ears in each replicate were used.

RESULTS AND DISCUSSION.—Results of experiment 1 are presented in table 1.

Neither malathion, regardless of dosage level, nor Pyrenone prevented an increase in ear infestation by the rice weevil. Within 1 month after treatment infestation increased from 37% to approximately 90% in all treatments. After 3 months' storage, infestation in the control had increased to 100%. Ears in the treated lots were 90% to 97% infested.

Statistically, only the Pyrenone-treated corn contained less kernel damage than occurred in the control after 2 months' storage. Damage in this treatment, however, rapidly increased after 2 months' storage and subsequent damage nearly equalled that in the control. At the end of 3 months' storage all treatment levels of malathion were very nearly equal and none differed significantly from the control. However, after 8 months' storage the 180 and 360 pounds per 1,000 bushels rates were significantly less damaged than the control. The 60-pound rate did not differ from the control. Kernel infestation in the control after 8 months' storage was 48%. Kernel infestation at the higher dosage levels of malathion was approximately 25%. Although the infestation level increased between 8 and 10 months' storage, both of the higher rates of malathion continued to be significantly better than the control. Kernel damage in the control after 10 months' storage was 72% as compared with approximately 30% for the two higher dosage levels of malathion, 53% for the lower dosage rate, and 60% for the Pyrenone-treated corn.

The value of Pyrenone grain protectant and two dosage levels of malathion for control of insects in shelled corn is shown by data presented in table 2. All treatments resulted in significantly less damage than in the control for a storage period of 6 months. However, between 6 and 9 months of storage, infestation in the Pyrenone treatment increased by 6 times. During the same time infestation also increased in both malathion treatments but not so much as in the Pyrenone treatment. Differences between the percentage of damaged kernels in both malathion treatments and the control were significant throughout the 11 months' storage period. However, when the condition

Table 2.—Protective value of malathion and pyrethrum applied to shelled corn against insect damage in storage September, 1958–August, 1959.

Dust Treatment (lb./1,000 bu.)	Per Cent Kernel Damage and Numbers of Adult Rice Weevils in 150-Gram Samples After[a]												Per Cent of Damage After 11 Months Caused by:	
	1 Month			2 Months			4 Months			Kernel Damage after Months Shown				
	Kernel Damage	Number Alive	Number Dead	Kernel Damage	Number Alive	Number Dead	Kernel Damage	Number Alive	Number Dead	6	9	11	Angoumois Grain Moth	Rice Weevil
1% malathion, 60	2.2	1	2	5.0	1	19	7.7	0	20	6.4	17.3	46	5	95
1% malathion, 180	2.4	1	1	4.3	0	6	6.7	0	5	5.3	8.7	20	0	100
Pyrenone grain protectant, 100	2.5	1	0	7.0	1	2	8.9	1	2	8.5	47.3	63	13	87
Control	3.3	3	0	21.4	18	9	27.4	37	3	33.0	92.7	98	[b]	[b]
L.S.D. at 5% level				3			3			3	16	19		

[a] Initial kernel damage at time of storage was 1.5%; initial grain moisture was 16.5%; the grain moisture in all replicates gradually decreased to approximately 14% at the termination of the test.

[b] Impossible to differentiate damage by species owing to excessive amount of kernel damage.

Table 3.—Effectiveness of malathion for protection of stored snapped corn from rice weevil damage.[a]

MALATHION TREATMENT[b] (P.P.M.)	EARS (%) INFESTED AFTER MONTHS SHOWN			KERNELS (%) DAMAGED AFTER MONTHS SHOWN			AFTER 11 MONTHS	
	6	9	11	6	9	11	Grain Moisture[c]	Grain Weight per Bushel (lb.)
1% malathion dust, 10.5	48	96	97	5	24	34	13.2	51.5
1% malathion dust, 14	76	95	96	8	30	36	13.4	51.3
1% malathion dust, 21	63	95	98	8	20	25	13.5	52.8
1% malathion dust, 42	70	90	98	5	15	23	13.5	53.3
Malathion E.C. on sawdust, 16[d]	45	94	99	3	16	27	13.5	52.5
Malathion E.C. on sawdust, 32[d]	58	99	99	2	24	25	13.7	52.7
Control	81	99	100	7	39	53	13.4	48.0
L.S.D. at 5% level				4	9	10.5		

[a] Test conducted on corn of low initial infestation (0.2% kernel damage).
[b] See text for details.
[c] The grain contained 15.8% moisture at time of storage.
[d] One pint of sawdust was used per 20 bushels.

of the corn is considered the practical importance of the differences is not so great as the figures indicate. After 11 months 63% of the Pyrenone-treated grain was damaged, 46% in the 60-pound rate of malathion, 20% in the 180-pound rate of malathion and 98% in the control. The differences in damage between the lower and higher rates of malathion are significant at the 11-month examination period. Previous to 11 months there was no difference in amount of kernel damage between the two.

Test number 3 was designed to determine if malathion used on lightly infested snapped corn at time of storage could effect a practical degree of protection from the rice weevil for approximately 12 months of storage.

Factors such as temperature and relative humidity which influence progressive damage during storage were not altered.

Weevil damage during the first 6 months of storage increased many fold; however, owing to the extremely low initial infestation and the ensuing winter season only 7% of the untreated corn was infested after 6 months. None of the malathion dust treatments at the 6-month examination period was significantly less damaged than the control. However, both malathion treatments using emulsifiable concentrate adsorbed on sawdust were significantly less damaged than the control. After 9 months' storage damage in the untreated corn had increased to 39%. At the same time kernel damage in all malathion dust treatments averaged approximately 22% and all differed significantly from the check. The 240-pound rate was significantly better than the 60- and 80-pound rates. Protection from insect damage in the malathion-sawdust treatments compared favorably with approximately equivalent amounts of malathion derived from a dust source. After 11 months' storage, damage in the control was 53%. Damage in the malathion dust treatments was 34%, 36%, 25% and 23% for the 60-, 80-, 120- and 240-pound rates, respectively. All were significantly less damaged than the control. Damage in the 240-pound rate was significantly less than in the 60- and 80-pound rates (table 3).

Information from these studies makes it evident that malathion applied to snapped or shelled corn offers some measure of protection from damage in storage by insects. However, under Louisiana conditions of high levels of infestation, favorable temperature for insect development during much of the storage period, high relative humidity and grain moisture prevent adequate insect protection of stored corn with malathion. Application of heavy rates of malathion increased the degree of protection offered the grain over lower rates. However, regardless of the rate used, only partial protection was obtained. Protection of shelled corn was greater than that obtained for snapped corn using equivalent rates of malathion. This difference in control is probably explained on the basis of better insecticide coverage of the shelled than of the snapped corn. For a storage period of not more than 4 to 6 months during the fall and winter season protection of shelled corn from excessive damage caused by stored-grain insects might be expected from the use of malathion.

One of the main objections offered by farmers to the use of malathion dust on corn is the presence of the dust itself on the kernels or ears. The data presented in table 3 show that equal protection may be possible by using malathion emulsifiable concentrate adsorbed on sawdust or some similar material and applying this to the grain. This method eliminates the undesirable features encountered when a dust is used.

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Esterase Activity and Susceptibility to Parathion at Different Stages in the Life Cycle of Organophosphorus-Resistant and Susceptible House Flies[1]

WALTER S. BIGLEY and FREDERICK W. PLAPP, JR., *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Corvallis, Oregon*

ABSTRACT

Quantitative determinations utilizing colorimetric techniques were made on cholinesterase (ChE) and aliphatic-esterase (Ali-E) activity at different stages in the life cycle of a susceptible and two organophosphorus-resistant strains of the house fly (*Musca domestica* L.). The toxicity of parathion residues to larvae, pupae, and adults of a susceptible and parathion-resistant strain was also determined.

It was found that, as in the adults, levels of Ali-E activity were markedly less when the resistant flies were in immature stages. ChE levels were similar for all fly strains at the stages of the life cycle tested. Larvae and pupae of the parathion-resistant strain possessed a degree of resistance approximately equal to that present in adult flies. The variation in susceptibility of the different stages tested did not appear to be correlated with variations in the amount of either ChE or Ali-E present.

Levels of aliphatic-esterase (Ali-E) have been found to be lower in organophosphorus-resistant than in susceptible strains of the house fly (*Musca domestic* L.), whereas there are substantially no differences in levels of cholinesterase (ChE) activity between resistant and susceptible flies (Van Asperen & Oppenoorth 1959, Bigley & Plapp 1960).

Using a system of repeated backcrosses, Oppenoorth (1959) demonstrated that low Ali-E activity and mathion resistance in the house fly were dependent on one and the same autosomal gene. He also found that in a strain resistant to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) the gene responsible for low levels of Ali-E accounted for only part of the resistance present.

In vitro inhibition studies with parathion and paraoxon have shown that under certain conditions Ali-E activity is more susceptible to inhibition than is ChE (Bigley & Plapp 1960). However, *in vivo* experiments have shown that the pattern of inhibition of Ali-E is not well correlated with the symptoms of toxicity, whereas ChE inhibition closely follows the poisoning symptoms exhibited by insecticide-treated flies (Plapp & Bigley 1961). In studies in which tri-*o*-cresylphosphate (TOCP) was employed as a selective inhibitor of the ali-esterase, Stegwee (1959) showed that inhibition of this enzyme was apparently not related to the toxic action of TEPP to house flies.

Since lower levels of Ali-E activity and resistance to organophosphorus insecticides appear to be related in adult house flies, studies of esterase activity in the immature stages were undertaken. Levels of ChE and Ali-E were determined in larvae, pupae, and female adults of susceptible, parathion-resistant, and malathion-resistant strains of house flies. The toxicity of parathion to the same stages of the susceptible and parathion-resistant colonies was also measured in an attempt to determine if there were any correlation between levels of either esterase and insecticide susceptibility.

EXPERIMENTAL PROCEDURE.—*Esterase Activity Determinations.*—Levels of ChE and Ali-E were determined daily in larvae and pupae of susceptible (Orlando Regular), parathion-resistant (Tropical P), and malathion-resistant (Grothe) strains of flies. The origin of these strains has been described (Wilson et al. 1959). Based on topical bioassays at the time this work was undertaken, the LD-50 of parathion was about five times greater for the Tropical P than for the Orlando Regular strain. The Grothe strain was about 30 times more resistant to malathion than the susceptible strain. A low order of cross-resistance to malathion was present in the Tropical P strain, and to parathion in the Grothe strain, although accurate measurements, of the cross-resistance present were not made at the time the study was started.

Larvae, pupae, and adults from the three colonies were obtained from eggs collected at the same time. Groups of 10 larvae, pupae, and adults were weighed at each sampling to determine their average weight, with the exception of 1-day-old larvae, of which 0.4-ml. samples, considered to be equivalent to 400 mg., were used. Methyl *n*-butyrate (Meb) at 4×10^{-3} M and acetylcholine bromide (Ach) at 2.5×10^{-3} M were used as substrates for determining Ali-E and ChE activity, respectively. The results were expressed as μM of substrate hydrolyzed per 15 minutes per gram of tissue. Whereas only heads (for ChE) and bodies (for Ali-E) were used in determinations of adult esterase activity, activity of either esterase was based on the weight of whole flies. All analyses were based on a minimum of three replications. Determinations were made by the colorimetric method of Hestrin (1949), and the reagents used and details of analysis have been previously described (Bigley & Plapp 1960).

To obtain 1-day-old larvae, eggs were collected from the different colonies and placed in petri dishes on filter paper moistened with distilled water. The dishes were kept in a fly-rearing room at a temperature of 75° to 80° F. Larvae were collected about 12 hours after hatching. Older larvae were obtained by allowing them to de-

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velop in standard fly media from which they were removed as needed. Homogenates for analysis were made to a concentration of five larvae per milliliter, and 1 milliliter of the homogenates was assayed, with the exception of the newly emerged larvae of which 40 milligrams of larvae were analyzed.

Pupae were obtained from the same rearing cans as the larvae and were rinsed in distilled water prior to analysis. Homogenates were prepared at a concentration of one per milliliter for ChE determinations and two per milliliter for Ali-E determinations. As soon as emergence started the rearing cans were placed in holding cages for 24 hours and then removed. Adults collected at 24 hours were considered to be 1 day old. Older adults were tested at 2, 7, and 14 days. Only females were used. Homogenates were prepared to contain concentrations of one head or two bodies per milliliter for ChE and Ali-E determinations, respectively.

Results of Esterase Activity Determinations.—Figure 1 shows the levels of ChE and Ali-E activity in larvae, pupae, and adult females of susceptible, malathion-resistant, and parathion-resistant colonies of house flies. ChE activity was high in 1- to 2-day-old larvae of all the colonies and then dropped markedly and remained relatively constant in later larval stages. In the pupae an increase in ChE activity occurred with age. One-day-old female adults were higher in ChE activity than older ones, in which activity of this enzyme remained relatively constant.

Levels of Ali-E activity were lower in both resistant colonies in all stages of the life cycle tested when compared with the normal colony. However, the pattern of Ali-E activity was similar in all the colonies within the life stages tested. Pupal Ali-E activity decreased with age in all the colonies except the oldest pupae from the normal colony, in which Ali-E activity rose considerably. This may have been because the normal pupae were sampled as they neared emergence. An additional pupal age was analyzed in the malathion-resistant colony since the onset of emergence occurred a day later with this group. One-day-old female adults were higher in Ali-E activity than older flies, in which activity of this enzyme, as with ChE, remained relatively constant.

Female adult Ali-E activity was lowest in the malathion-resistant colony. In a previous paper Bigley & Plapp (1960) reported that Ali-E activity was lowest in adult females of the parathion-resistant colony. Since the colonies were maintained without further pressuring, a considerable decrease in tolerance to parathion had occurred in the parathion-resistant strain prior to these

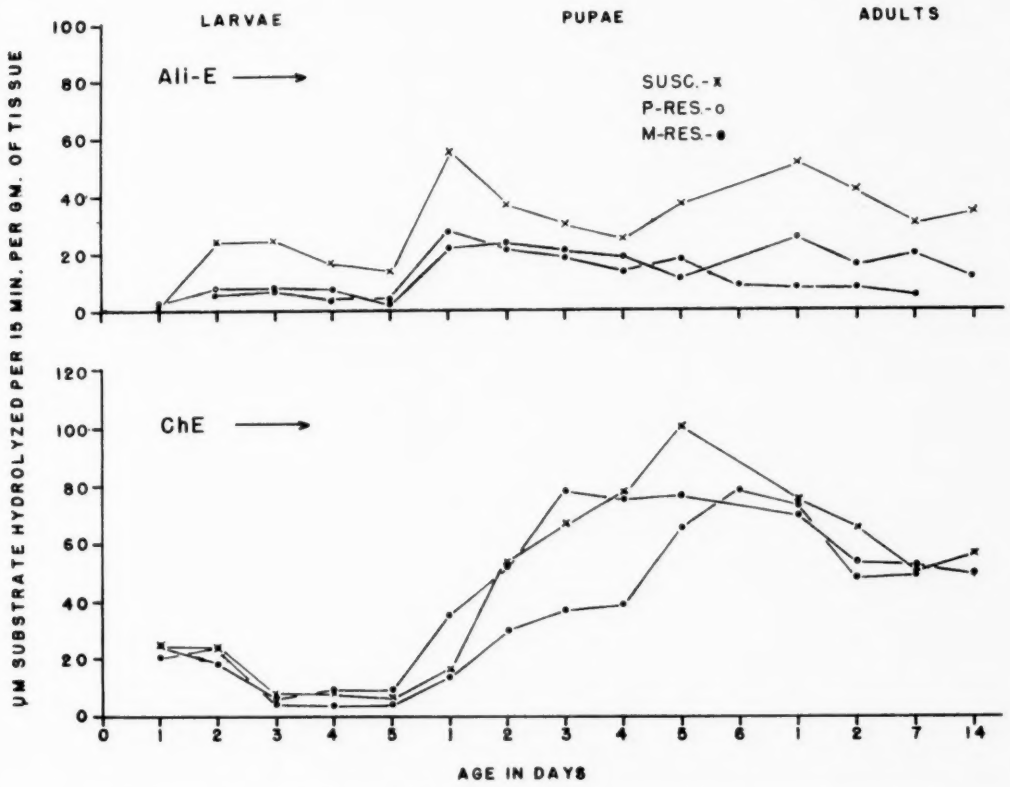

FIG. 1.—Levels of ChE and Ali-E activity in larvae, pupae, and female adults of susceptible, parathion-resistant, and malathion-resistant house flies.

Table 1.—Esterase activity and susceptibility to topically applied parathion of normal and parathion-resistant 3-day-old female house flies on the dates indicated, 1959.

Colony	Per Cent of Substrate Hydrolyzed on—				Parathion for LD-50 (μg./fly)	
	3/16		8/23			
	ChE	Ali-E	ChE	Ali-E	3/16	8/23
Normal	50	43	45	48	0.012	0.012
Parathion-resistant	46	19	46	36	.06	.04

studies. Table 1 shows the susceptibility of the normal and parathion-resistant house fly strains to parathion and ChE and Ali-E activity when first obtained and just prior to these studies. As susceptibility to parathion increased in the parathion-resistant colony, Ali-E activity also increased. This result indicates a close relationship to parathion susceptibility and the level of Ali-E activity in the parathion-resistant strain. No comparable data are available for the malathion-resistant strain.

Bioassay Determinations.—Larvae, pupae, and adults of the susceptible and parathion-resistant colonies were tested for their susceptibility to parathion. The methods of collecting flies in the different stages were the same as those described for the esterase determinations. Larvae younger than 3 days were not tested because of the difficulty in keeping them alive for the duration of the experiments. Female flies only were used in the adult bioassays.

The stages of the two colonies studied were exposed to Whatman No. 1 filter paper 9 cm. in diameter and treated with 0.5 ml. of acetone solutions containing the desired amounts of technical-grade parathion. This amount of acetone solution saturated the filter paper without runoff. The treated papers were allowed to air dry for approximately 1 hour. They were then placed in petri dishes and 0.5 ml. of a 10% sucrose solution for adult treatments was pipetted onto each paper. Addition of the sucrose solution was necessary in the treatment of adults in order to prevent mortality of the control group. Larvae, pupae, or female adult flies were then placed on the treated papers and the petri dishes covered. Replicates of 30 flies were run at each parathion concentration. All treated insects were held in a holding room at 75° to 80° F. Larvae were held in covered cardboard boxes to minimize light intensity in the holding room.

Larvae and pupae were exposed to treated filter paper for 24 hours and then transferred to clean petri dishes containing moistened filter paper. Insects in immature stages were kept in these dishes until adult emergence was complete.

Adult emergence of treated larvae and pupae was used as the criterion of parathion toxicity. Twenty-four-hour mortality figures were not obtained for larvae because much higher concentrations of parathion than those reported here would not kill them, although normal pupation and emergence were prevented. Survival figures were recorded for adult females after 24 hours of exposure to treated papers. Controls for insects in all stages were prepared by treating filter papers with 0.5 ml. of acetone and, after drying, adding a similar amount of water or a 10% sucrose solution.

Table 2 shows the susceptibility of larvae, pupae, and adult females of normal and parathion-resistant house flies to parathion. Larvae of the parathion-resistant strain were from 3 to 13 times less susceptible to parathion than insects of the same ages in the normal colony. Larval susceptibility of the normal colony to parathion decreased with age. Four-day-old larvae of the parathion-resistant strain appeared to be less susceptible to parathion than 3- or 5-day-old resistant larvae. However, the results with the parathion-resistant larvae were variable compared with the normal larvae. As the data for both colonies show, large ranges of insecticide concentrations were required to produce substantial differences in mortalities. Affected larvae produced malformed pupal cases, elongated and larval-like in appearance. Larvae and pupae treated with parathion did not show a significant difference in time required for emergence compared with untreated controls. Maximum emergence of most of the flies occurred within 24 hours after the onset of emergence.

Pupae of the resistant colony were at least 100 times less susceptible to parathion than pupae of the normal colony. At dosages of parathion up to 1.0%, no less than 60% survival was obtained. Although pupae from the parathion-resistant colony gave a high degree of emergence, even with high concentrations of parathion, the flies usually died within 2 to 3 hours after emerging. This mortality was apparently a result of contact with the treated pupal cases during or after emergence. Flies emerging from normal pupae treated with parathion remained alive at least 24 hours except at the highest

Table 2.—Toxicity of parathion residues to normal and parathion-resistant larvae, pupae, and adult female house flies.

Concentration of parathion (%)	Survival of Susceptible Insects at Indicated Days (%)					Concentration of Parathion (%)	Survival of Parathion-Resistant Insects at Indicated Days (%)				
Larvae											
	3		4		5		3		4		5
0.00006	100		83		100	0.0002	80		—		—
.0001	70		70		70	.0003	57		77		53
.0002	27		20		50	.0006	40		57		37
.0003	30		20		20	.001	47		70		40
.0006	23		23		27	.002	53		67		40
.001	0		0		10	.003	—		43		33
Pupae											
	1	2	3	4			1	2	3	4	
0.001	100					0.1	73	97	77	100	
.002	—					.2	73	87	73	83	
.003	53					.3	70	87	87	77	
.006	10	100	97	100		.6	67	70	67	80	
.01	0	50	30	57		1.0	67	70	60	80	
.02		3	3	10							
.03		0	0	3							
.06				3							
Adults											
	1	2	3	6	10		1	2	3	6	10
0.00006	97	100	100	83	87	0.0003	83	—	97	—	—
.0001	97	97	77	67	43	.0006	73	97	83	90	—
.00015	53	70	80	3	0	.001	77	87	73	80	77
.0002	0	23	20	0	0	.002	47	43	40	43	37
						.003	27	20	20	—	20
						.006	0	0	0	—	—

Table 3.—Resistance levels and ChE and Ali-E activity in two colonies of resistant house flies compared with susceptible flies.[a]

Colony	Generation	Esterase Activity of Susceptible Flies (%)		Resistance Ratio at LD-50 Compared with Susceptible Flies[b]
		ChE	Ali-E	
Malathion-resistant	0	90	58	10
	7	95	42	27
	10	98	37	44
Parathion-resistant	0	90	40	5
	7	95	28	14
	8	107	25	13

[a] Resistant strains pressured each generation.
[b] Based on topical applications of insecticides.

concentrations used. The susceptibility of normal and parathion-resistant pupae to parathion decreased with age.

Parathion-resistant female flies were about 10 times less susceptible to parathion than normal females up to 3 days old. Normal females became more susceptible to parathion with age, whereas insecticide susceptibility did not change much in parathion-resistant female adults.

Further Development of Resistance.— More recently the parathion-resistant and malathion-resistant colonies have been maintained by pressuring each generation of adults. The flies were pressured by feeding newly emerged adults with insecticide-impregnated food, consisting of equal amounts of powdered milk and sugar.

The degree of resistance in these two colonies, determined by topical applications of the insecticides, as well as ChE and Ali-E activity, was ascertained periodically. Table 3 compares the resistance levels and ChE and Ali-E activity through several generations of pressuring in the resistant colonies with those in the susceptible colony. Resistance to malathion increased from 10 to 44 times when compared with a phosphate-susceptible colony at the LD-50 level through 10 generations of pressuring. Resistance to parathion increased from 5 to 14 times in eight generations of pressuring.

As in previous experiments, levels of ChE activity were similar in all the strains, whereas Ali-E activity was markedly lower in both resistant strains. Depressed Ali-E activity is directly correlated with the degree of resistance in both malathion- and parathion-resistant fly strains. The parathion-resistant strain, however, is much lower in Ali-E activity than the malathion-resistant, but the degree of resistance is less in the parathion-resistant strain.

DISCUSSION.—Levels of ChE and Ali-E activity did not appear to be correlated with parathion susceptibility at any of the life stages of the fly colonies tested. If the levels of esterase activity and insecticide susceptibility were related, one would expect either an increase or decrease in susceptibility with a rise or fall in activity of either esterase. This situation would be especially true with Ali-E activity, since it has been shown that lower levels of Ali-E activity are associated with phosphate resistance in house flies. These studies indicate that resistance and lower levels of Ali-E activity are related, and that this relationship exists in flies in the immature stages as well as the adult stage. However, the levels of Ali-E activity in flies within a particular life stage do not apparently influence the susceptibility of the insects in that stage to parathion. This is true with susceptible as well as parathion-resistant flies.

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Factors Influencing Pink Bollworm Pupation and Moth Emergence From Overwintering Larvae in Central Texas[1]

L. C. Fife, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2,3]

Abstract

Time and rate of moth emergence of the pink bollworm (*Pectinophora gossypiella* (Saunders)) from overwintering larvae in two environments over a 6-year period at Waco, Texas are reported. Two major peaks of emergence occurred in 5 of the 6 years from infested open cotton bolls on the soil surface and in 4 years from buried bolls. Usually major peaks occurred 16 to 25 days after an inch or more of rainfall on 1 or more days. Smaller peaks occurred within the same intervals after approximately 0.5 inch of precipitation. Usually major peaks were higher and extended over a longer time as the amount of precipitation on 1 to several days increased. As the temperature increased, the number of days between heavy rainfall and high peaks decreased. Burial of infested open bolls resulted in earlier moth emergence than that from bolls on the soil surface. Also, above-normal spring rainfall resulted in earlier emergence than that occurring in dry springs, from both environments, and consequently a higher percentage of the moths died before suitable fruiting forms on cotton were available for propagation. In general, the diapause period was longer than average and was accompanied by a higher mortality during drought conditions from April through June. As the planting date was delayed, so was the fruiting date, and moth emergence to infest cotton was greatly reduced in both environments.

Because of heavy infestations of the pink bollworm (*Pectinophora gossypiella* (Saunders)) in central Texas in recent years, investigations have been continued on the biology and habits of the pest, with emphasis on the major factors influencing its carryover from one cotton crop to the next. Previous work was reported by Fife et al. (1957). In November 1952 investigations on the overwintering of the insect were started at Waco, Texas. The more important factors influencing the time and rate of pupation and moth emergence from two environments over a 6-year period are discussed herein.

The insect overwinters in the mature larval stage. A large majority of the larvae remain inside the seed of open cotton bolls that are left in the field after harvest. These larvae complete development during the next spring and summer, but wide variations in time and rate of pupation and moth emergence frequently occur.

Method.—Cages with removable screen-wire pyramid-type covers, as described by Shiller (1946), were used to determine the time and rate of moth emergence from overwintering larvae.

Moth emergence from infested open bolls was observed in three environments, as follows: (1) Bolls placed on the soil surface November 15 and buried 2 inches on February 15, (2) bolls buried 2 inches on November 15, and (3) bolls held on the soil surface throughout the experiment. Since the time and rate of emergence for the first two groups were essentially the same, these records were combined for this study, although the winter survival percentage was considerably higher in the February-buried group.

On November 15 of each year, 3½ pounds of bolls were placed in each cage. There were 20 cages per treatment in 1952, 1953, and 1954; 15 in 1955; and 10 in 1956 and 1957. After the bolls were thoroughly mixed, samples were taken at random from each collection and examined to determine the initial larval population per cage.

The removable covers, equipped with glass-jar moth traps, were placed on the cages on March 31 of each year; thus the bolls in the box-like open base were exposed to essentially outside temperature and moisture conditions during the fall and winter months. Moth emergence was recorded almost daily from April 1 until completed.

The results are based on the emergence of 5,466 moths from the buried bolls and 12,923 from those on the soil surface over the 6-year period, with yearly totals as given in table 2.

Records on temperature and rainfall were obtained from the U. S. Weather Bureau, located about 0.8 mile from the test plots. The daily mean temperatures, as shown in figures 1 and 2, were based on a 7-point moving average, dropping 1 day and adding 1 as the season advanced. Therefore, each daily mean included the 3 days before, the 3 days after, and the day on which it was plotted.

In figures 1 and 2 the percentage of the total emergence during each 7-day period was plotted on the last day of that interval. Daily emergence varied greatly and, consequently, the weekly totals showed more clearly the general trend of emergence throughout the season.

Results.—*Yearly Moth Emergence Patterns.*—The time and rate of moth emergence from surface bolls varied greatly during the 6-year period (fig. 1). Two major peaks of emergence occurred in all years except 1957.

Moth emergence patterns during 1953, 1954, and 1955 were similar. The first major peak during each of these years occurred on about the same date, ranging from April 28 to May 2. Furthermore, the dates of the second major peaks varied only 9 days, that is from June 2 to 11.

In 1956 and 1958 both major peaks occurred late in the season. In 1956, a dry season, emergence began early, on April 2, but the major part was greatly delayed, due to lack of sufficient moisture for pupation and moth emergence until after the heavy rainfall on May 1. Considerable emergence occurred in July and August. However, in 1958, emergence began on April 23, or approximately 1 to 3 weeks later than in the previous years. In 1957, a wet season, only one major peak of emergence was observed with approximately 75% of the total occurring during the 20-day period May 17 to June 8.

The highest percentage of moths emerged during the

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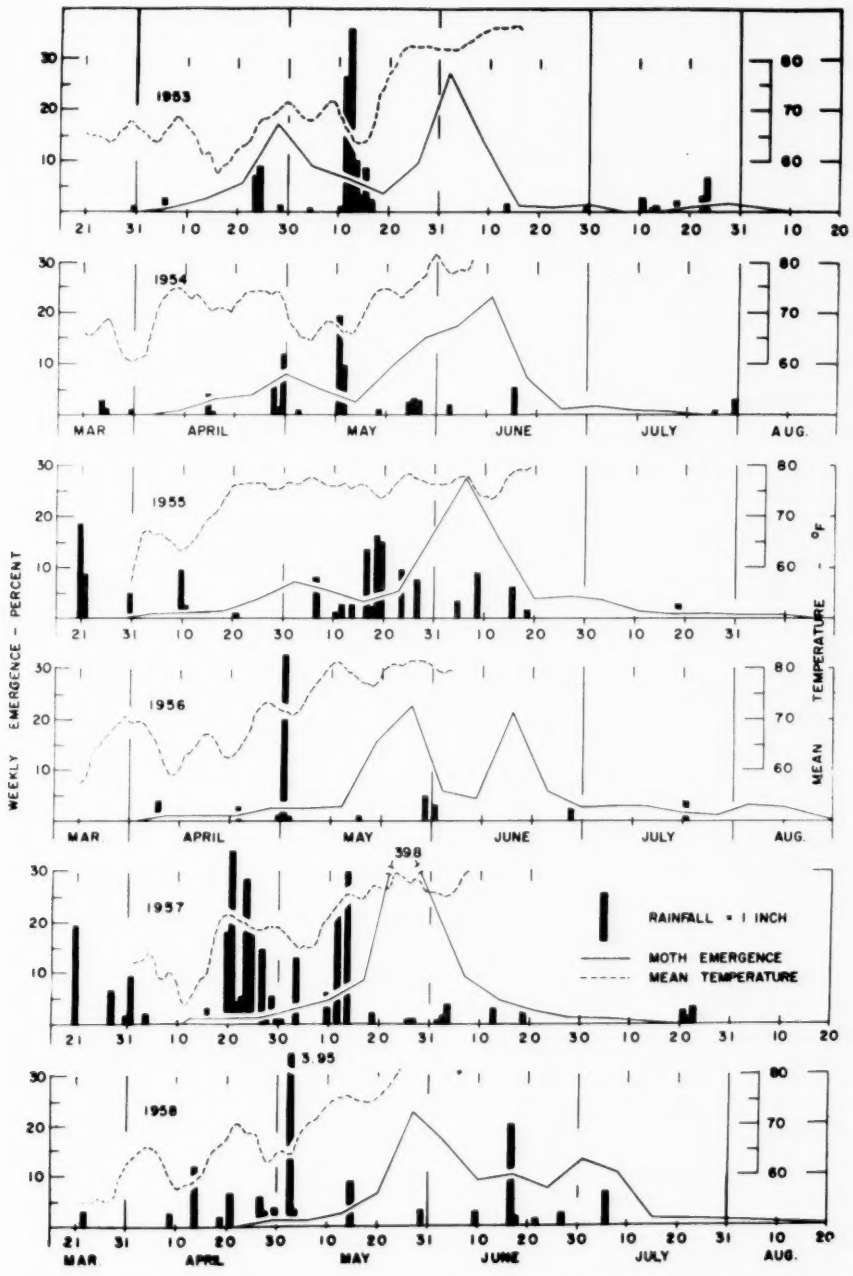

FIG. 1.—Pink bollworm moth emergence from infested open cotton bolls on the soil surface in relation to temperature and rainfall over a 6-year period.

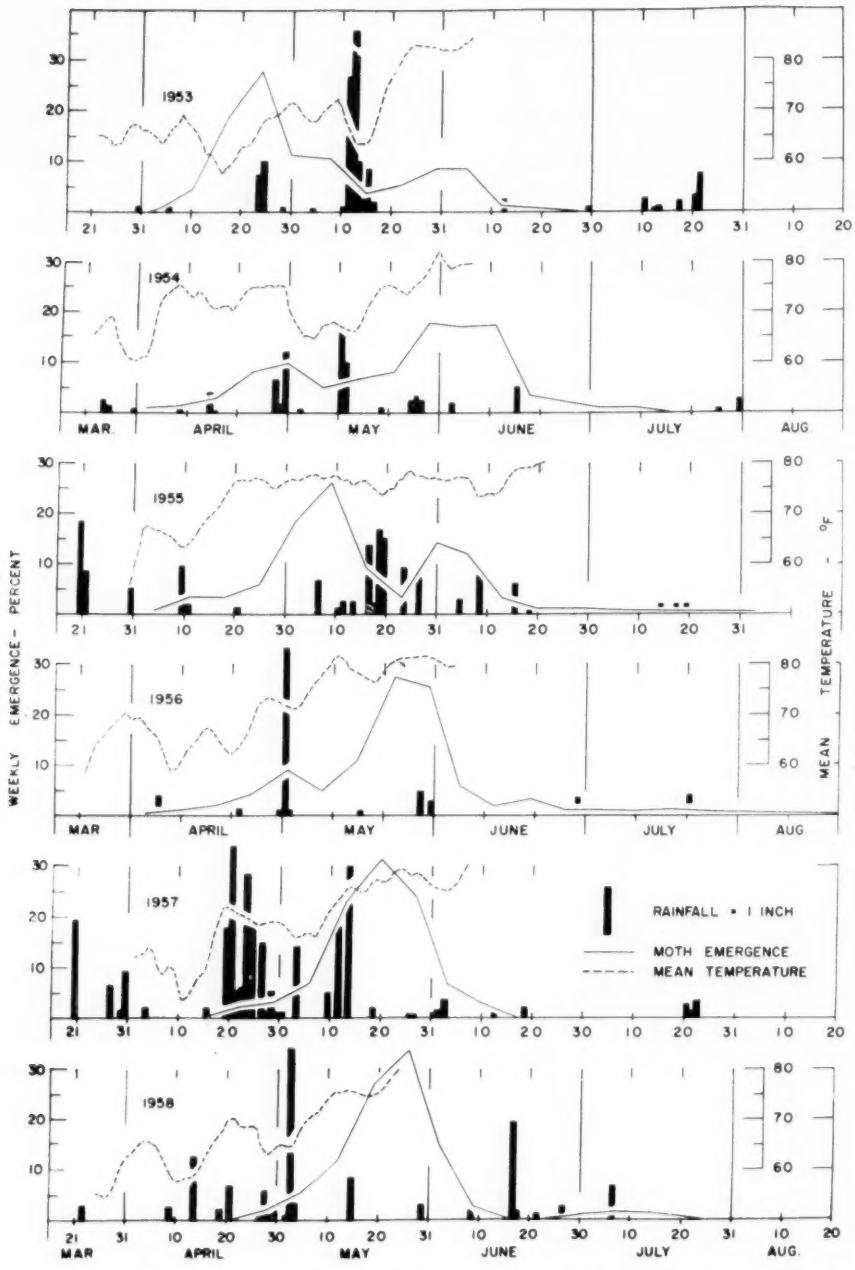

FIG. 2.—Pink bollworm moth emergence from infested open cotton bolls buried 2 inches in relation to temperature and rainfall over a 6-year period.

second peak when the first occurred early in the season, as in 1953, 1954, and 1955. The opposite was true when the first peaks of emergence occurred late in the season, as in 1956 and 1958. The period between two major peaks for any 1 year varied from about 20 days in 1956 to about 42 days in 1954. The longest emergence period, 145 days, extended from April 1 to August 24, 1955. The shortest period of 96 days was recorded in 1957, a wet season that extended from April 11 to July 16. Emergence terminated in August during 4 of the 6 years.

With the exception of 1958, the number of major peaks of moth emergence from buried bolls for the various years was the same as that from bolls on the soil surface (fig. 2).

From 1953 to 1956, inclusive, the first major peaks occurred within a 15-day period, April 24 to May 9. However, the date of the second major peaks varied within an 8-day period, May 22 to 30. For the different years the number of days between major peaks was essentially the same as from bolls on the soil surface—20 to 42 days. Only one major peak occurred in 1957 and 1958. The early emergence, considerably delayed in both years, began about 2 to 3 weeks later than in the previous years.

The rate of emergence from the buried bolls was earlier than from those on the soil surface. For example, on May 8, 15, 22, and 29 cumulative emergence over the 6-year period averaged 35%, 48%, 65%, and 83% from the buried bolls and 16%, 19%, 35%, and 53% from those on the soil surface, respectively (table 2). Also, emergence terminated earlier from the buried bolls and ranged from 48 days in 1957, a wet season, to 144 days in 1956, a dry season. Winter survival was lower in the buried environment, 5.6% compared with 26% on the surface environment.

Emergence in Relation to Temperature and Rainfall.—The wide variations in the yearly patterns of moth emergence were largely because of differences in temperature and rainfall during April, May, and June. Over the 6-year period 87% or more of the total emergence occurred during this period, regardless of the environment.

With two exceptions, major peaks of emergence in both environments occurred 16 to 25 days (depending on temperature) after 1 inch or more of rainfall on 1 to several days (figs. 1 and 2). The exceptions noted were the first major peaks in 1953 and 1954, when rainfall varied from 0.24 to 0.42 inch. These major peaks were usually higher and extended over a longer time as the rainfall on 1 to several days increased. Major peaks of emergence usually extended over about 15 to 25 days.

Actually, the apparent major peaks, based on weekly emergence, may have been composed of several smaller peaks, which also showed a relationship to temperature and rainfall. For example, the daily records of 1954 showed that in both environments three high peaks of emergence occurred that corresponded to the three periods of heavy rainfall (fig. 3). These three peaks from bolls on the soil surface occurred on May 21, June 1, and 11, or 24, 22, and 18 days after 0.85 inch or more of rainfall on 1 or more days (fig. 3-A). These three periods of rather heavy precipitation fell on April 27 to 30, May 10 and 11, and May 24 to 26. When the emergence was plotted on a weekly basis (fig. 1), the combined effect of the three periods of rainfall was an apparent continuous heavy second major peak, extending over a 25-day interval.

As the temperature increased, the number of days between heavy rainfall and high peaks of emergence decreased, in an essentially straight-line relationship. For example, at average temperatures of 69.5°, 74.0°, and 80.2° F., the intervals between rainfall and peak emergence were 24, 22, and 18 days. The results were about the same with buried bolls (fig. 3-B).

In 1953, the second major peak of emergence occurred on June 1 in both environments. This second peak occurred 21 days after 9.59 inches of rain fell over a 6-day period that began on May 11. Daily mean temperatures averaged 75.5° F.

A total of 1.12 inches of precipitation occurred on April 9 and 10, 1955, and the first major peaks of emergence occurred 22 and 23 days later, or on May 1 and 2 in the buried and surface bolls, respectively. Mean temperatures averaged about 73.4° F.

A rainfall of 3.34 inches occurred on May 1, 1956 and the first major peaks of emergence were observed 21 and 23 days later, or on May 22 and 24, in the surface and buried bolls, respectively. Less than 0.25 inch of rain fell 25 days prior to or after May 1. Daily mean temperatures averaged approximately 77.4° F. The second major peak in 1956 was observed on June 14 and 15 in the buried and surface bolls, respectively. This peak occurred 16 and 17 days after 0.77 inch of precipitation over a 3-day period, May 29 to 31, with daily mean temperatures averaging approximately 82.0° F. Less than 0.1 inch of rain fell 26 days before or after.

In 1957 only one major peak of emergence was observed in both environments. Heavy rainfall that totaled 20.21 inches occurred almost daily over the 24-day period, April 19 to May 13. The daily mean temperature averaged 64.2° F. in April, but increased rapidly after May 8. From 79% to 84% of the total emergence occurred during May from bolls in the two environments. This single peak, instead of two, was attributed to the prevailing weather. Low temperatures in April caused a delay in early-season emergence; and favorable temperatures and moisture conditions in May greatly accelerated moth emergence, bringing the usual two peaks together.

In 1958, only one major peak of emergence was observed from the buried bolls, whereas two such peaks occurred from bolls on the soil surface. Weather conditions during April and May of 1957 and 1958 were similar. During April of both years, rainfall was considerably higher and mean temperatures lower than for the same previous 4-year period (table 1). Also, precipitation during May was above normal both years. Mean temperatures averaged 64.2° during April of both years and did not exceed 70° until May 6, but increased rapidly after that date. In 1958 moisture in the buried environment was more conducive to pupation and moth emergence than that in the soil surface.

These data indicate that one major peak of emergence might be expected from infested bolls in both environments with below-normal temperatures in April, accompanied by frequent periods of heavy rainfall from about April 15 to May 15.

To determine the effect of rainfall on rate of emergence, 2 years with extremes of moisture conditions, 1956 and 1957, were compared. During April, May, and June of 1957 precipitation was considerably higher and mean

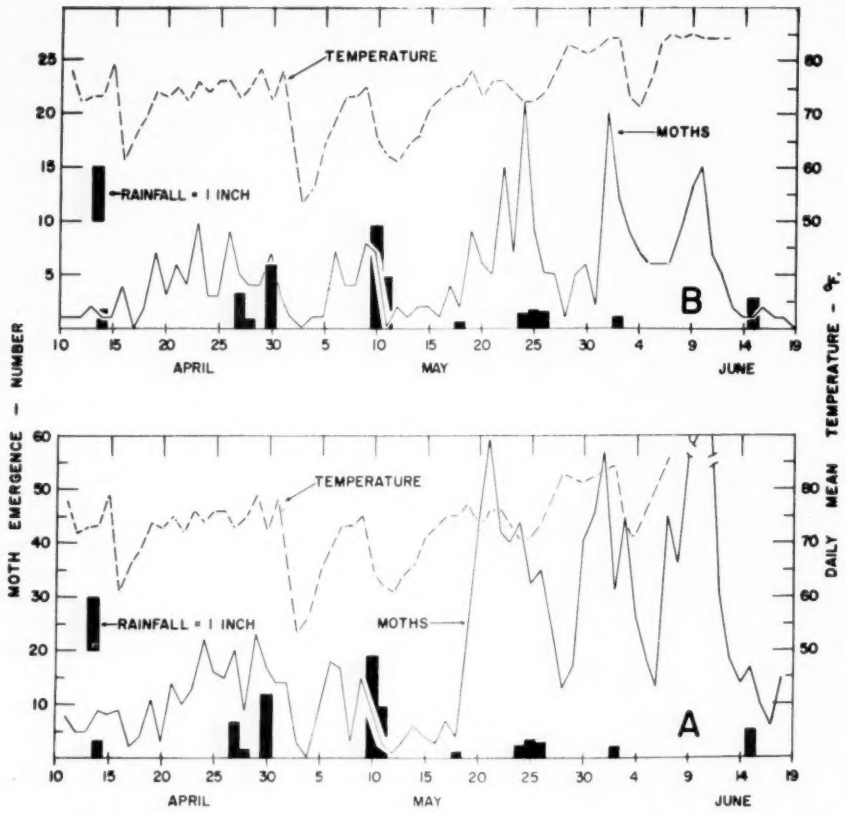

Fig. 3.—Daily pink bollworm moth emergence from infested open cotton bolls in relation to temperature and rainfall during 1954. (A) Bolls on soil surface; (B) bolls buried 2 inches.

temperatures lower than in 1956 (table 1). From May 22 to June 26, 1957, however, the weekly cumulative emergence was much higher in both environments than in 1956 (table 2). The cumulative emergence during this period was also higher in 1957 than all other years except 1953, when rainfall during May was much higher than in any of the following years (table 1). Heavy spring rainfall, therefore, resulted in earlier moth emergence in both buried and surface bolls.

Mean temperatures of 70° F. or below for 1 or more days greatly reduced the daily rate of moth emergence in both environments (fig. 3).

Emergence in Relation to Planting Date and Plant Fruiting.—Biological studies indicate that pink bollworm moths live, on an average, for 2 weeks or less during the early squaring period. Most of the eggs are laid during the first week of moth life. The eggs hatch in 4 to 5 days. Very few larvae develop in squares that are less than 10 days old when infested. Thus, only a few larvae survive from eggs laid by moths that emerge 3 or 4 days prior to the appearance of the first squares. Emergence before this date is considered suicidal.

Table 1.—Rainfall and mean temperature during March, April, May, June and July over a 6-year period.[a]

Year	Rainfall (inches)					Mean Temperature (° F.)				
	March	April	May	June	July	March	April	May	June	July
1953	3.21	2.04	9.72	.27	1.72	62.8	64.6	73.3	85.6	84.8
1954	.86	2.50	3.87	.79	.37	57.1	72.0	71.3	83.1	88.8
1955	3.20	1.30	7.61	2.01	.39	60.0	70.4	76.2	78.8	84.9
1956	.04	.65	4.25	.37	.34	59.8	66.4	78.2	83.8	87.5
1957	5.58	13.37	7.60	1.03	.61	56.3	64.2	73.6	81.2	87.4
1958	.96	3.60	5.58	2.72	.68	51.9	64.2	75.0	82.4	85.8
Normal	2.94	3.97	4.15	3.19	1.94	58.4	66.8	74.5	81.8	85.0

[a] Data from U. S. Weather Bureau.

Table 2.—Per cent cumulative pink bollworm moth emergence at weekly intervals over a 6-year period in two environments.

Date	From Bolls on Soil Surface							From Bolls Buried 2 Inches						
	1953	1954	1955	1956	1957	1958	Average	1953	1954	1955	1956	1957	1958	Average
April 10	2	2	1	1	0	0	1	5	3	3	2	0	0	2
17	5	5	2	2	1	0	3	23	6	6	4	0	0	7
24	16	9	6	3	2	0	6	51	15	12	8	3	0	15
May 1	30	17	12	5	3	1	11	62	25	28	17	7	4	24
8	38	22	18	7	7	2	16	72	30	53	22	19	13	35
15	44	25	22	16	14	5	19	76	36	66	33	44	32	48
22	50	36	26	34	45	16	35	81	46	69	60	70	61	65
29	67	51	42	50	74	36	53	90	63	82	86	92	87	83
June 5	88	69	67	55	88	51	70	99	81	94	92	97	97	93
12	97	90	83	66	94	61	82	99	96	98	94	100	98	98
19	98	97	89	81	97	70	89	99	99	99	97	—	98	99
26	98	98	93	86	99	89	94	100	99	99	98	—	98	99
Moths Emerged:	1,248	1,500	5,699	1,225	1,095	2,156	—	1,319	356	2,555	816	134	286	—
Survival (%)[a]	25.2	17.2	55.8	9.8	29.5	38.7	26.0	11.6	2.0	12.5	3.3	1.8	2.6	5.6

[a] Based on spring and summer moth emergence from the fall population.

Field observations in this area showed that the first squares began to appear on the earliest planted cotton about May 15. The weekly records of cumulative emergence show that a high percentage of the emergence was suicidal (table 2). Cumulative moth emergence before May 15 from bolls on the soil surface ranged from 5% to 44%, or an average of 19%. With buried bolls, however, 32% to 76%, or an average of 48%, of the total emergence was suicidal. In the College Station area of Texas, Brazzel & Martin (1959) reported that about 10% of the total emergence from open bolls on the soil surface took place before cotton fruit was available for food during 1953 and 1954.

Observations were made in 83 fields over a 3-year period on the relation between planting, squaring, and blooming dates. Cotton planted on April 2, 16, 29, May 9, 16 and 24, began to square, on an average, on May 15, 22, 29, June 5, 12, and 19, respectively. Moth emergence to infest cotton on the above planting dates was approximately 52%, 35%, 17%, 7%, 2% and 1% from buried bolls and 81%, 65%, 47%, 30%, 18%, and 11% from bolls on the soil surface, respectively. Consequently, as the planting date was delayed, the fruiting date was likewise delayed, and moth emergence to infest cotton was greatly reduced in both environments. For many years field observations have shown that the earliest planted fields in any given area are usually more heavily infested during the early fruiting period. However, extremely late planting is not considered practical in central Texas.

However, the data emphasize the importance of early and deep fall burial of the crop residue as a practical method of pink bollworm control in central Texas, since winter survival and moth emergence to infest cotton are greatly reduced under such conditions. The data also suggest the possibility that considerable control might be obtained in irrigated areas with a long growing season by delaying the preplanting irrigation and planting date of cotton.

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Effect of Feeding *Bacillus thuringiensis* to Caged Layers for Fly Control[1]

Edward C. Burns, B. H. Wilson and B. A. Tower, *Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge*

Abstract

Commercially prepared spore powders containing 3×10^9, 25×10^9 and 47×10^9 spores of *Bacillus thuringiensis* Berliner per gram were fed in the ration of caged layers. There was considerable variation in fly (*Musca domestica* L.) control obtained in the droppings when the different formulations were fed at the same spore levels. A reduction in feed consumption, body weight and egg production of caged layers was noted when the most effective material (Rohm & Haas) was fed in the ration at and above 53×10^6 spores per gram of feed.

The caged-layer method of egg production offers many advantages to the poultryman; however many problems are encountered. The favorable house fly, *Musca domestica* L., breeding environment found around caged-layer installations often results in serious fly problems. Although the use of chlorinated hydrocarbon and organic phosphate insecticides have been very helpful in keeping flies under control, development of resistance to many of the materials has made development of other methods

of control necessary. Briggs (1959, 1960) and Harvey & Brethour (1960) have reported inconsistent results obtained from feeding *Bacillus thuringiensis* Berliner in the ration of caged layers for fly control. This study was undertaken to evaluate the relative effectiveness of several commercially prepared spore powders for house fly control and to determine the effect of the treatments on layer performance.

PROCEDURE.—Single-comb white leghorn hens weighing an average of $3\frac{3}{4}$ pounds each were used in the tests. In laboratory studies, birds were housed in a large room in which the temperature was maintained at approximately 80° F. They were placed in a 24-bird laying cage as described by Burns et al. (1959) and were separated into four groups each consisting of five treatments and a check. Individual feed troughs and dropping pans were provided for each bird. Continuous lighting was obtained by suspending a 60-watt bulb 4 feet above the birds at each end of the battery. After the birds had received the treatments for 1 week, the dropping pans were cleaned and droppings were allowed to accumulate for approximately 1 week at which time droppings from individual birds were placed in 1-gallon fiber ice cream boxes with screened tops. Definite numbers of house fly eggs from the Orlando susceptible strain were placed on the droppings. The boxes containing the droppings and fly eggs were held at 80° F. for 3 weeks after which the number of flies which emerged was determined.

The spore concentrates were pre-mixed, in a small amount of feed and then blended in 25-pound lots using a small ribbon blender. Oyster shell and grit were provided free choice.

In the first tests Rohm & Haas[2] and Stauffer[3] powders containing 25×10^9 and 3×10^9 spores per gram, respectively, were used. The spores were fed in the concentrations shown in table 1.

Since it was desirable to obtain more information on the effect of the treatment on food consumption, egg eproduction, and body weight, the Rohm & Haas formulation was fed at 53×10^6 spores per gram of feed for 58 days to 29 caged layers housed in a standard laying cage installation under field conditions. An equal number of similar birds on the same ration minus the treatment served as checks. Results of this study are shown in table 2.

The Rohm & Haas formulation was evaluated under controlled laboratory conditions in two other tests to determine the lowest level of spores that could be fed to obtain effective fly control. Concentrations used and per cent control obtained are shown in table 3.

Table 1.—Per cent fly control in droppings from caged layers fed *Bacillus thuringiensis* in their ration.

Treatment	Spores/G. of Feed	Average Feed Consumed per Bird per Day (grams)	Average Spores Consumed per Bird per Day (billions)	Per Cent Fly Control	
				Test 1	Test 2
0	0	96.4			
Stauffer[a]	53×10^6	95.2	5.0	29	14
Stauffer[a]	105×10^6	91.3	9.7	17	13
Rohm & Hass[b]	53×10^6	60.4	3.2	99	96
Rohm & Haas[b]	70×10^6	70.9	5.0	100	99
Rohm & Haas[b]	105×10^6	55.3	5.8	100	100

[a] 3 billion spores/g.
[b] 25 billion spores/g.

Table 2.—Effect of *B. thuringiensis*[a] in the ration of caged layers on feed consumption, egg production and body weight.

Spores per Gram of Feed	Average Feed per Bird per Day (grams)	Average Spores per Bird per Day (billions)	Per Cent Egg Production	Average Body Weight	
				Date	
				6/22	8/19
0	89.8	0	63.3	3.78	3.81
53×10^6	76.2	4	39.3	3.82	3.63

[a] Rohm & Haas material, 25 billion spores/g.

Table 3.—Per cent control of house flies in droppings from caged layers fed *Bacillus thuringiensis*.[a]

Spores per Gram of Feed	Average Fly Emergence		Per Cent Control	
	Test 4	Test 5	Test 4	Test 5
0	89.7	55.5		
2.2×10^6	86.0	56.2	4	0
4.4×10^6	62.5	65.5	30	0
8.8×10^6	46.2	47.2	48	14
17.6×10^6	26.5	27.0	70	51
35.2×10^6	13.6	21.0	84	62

[a] Rohm & Haas material, 25 billion spores/g.

Table 4.—Average number of house flies completing development (egg to adult) in droppings from caged layers fed *Bacillus thuringiensis*[a] in their ration.

Spores per Gram of Feed	Average Fly Emergence	Per Cent Control
0	72.2	
70.5×10^6	85.3	0.0
88.1×10^6	55.6	21.6
105.8×10^6	72.5	0.0
132.2×10^6	65.0	9.9
141.9×10^6	59.2	19.3

[a] Bioferm Corporation material, 47 billion spores/gm.

In the final test a nonformulated spore powder containing 47×10^9 spores per gram was used in the ration. This material was prepared by Bioferm Corporation, Wasco, California and contained no wetting agents, dispersants or other diluents. Results obtained from this test are shown in table 4.

RESULTS AND DISCUSSION.—Considerable variation in fly control was obtained using equivalent spore concentrations from the different commercial sources. When fed at the same dosage in the ration of caged layers, the Rohm & Haas material was more effective. As shown in tables 1 and 3, averages of 97% and 73% fly control were obtained by feeding bacillus prepared by Rohm & Haas in the ration at 53×10^6 and 35×10^6 spores per gram of feed. This rate is higher than that reported necessary by Harvey & Brethour (1960) but lower than that found necessary by Briggs (1960). Feed consumption and egg production were

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[2] Furnished by Rohm & Haas Company, Philadelphia 5, Pa.
[3] Furnished by Stauffer Chemical Company, Mountain View, Calif.

reduced considerably when Rohm & Haas powder was incorporated in the ration at the rate of 53×10^6 viable spores per gram of feed (tables 1 and 2). Feed consumption was not affected when the Stauffer powder was fed at the same rate (table 1). As shown in table 4, no appreciable fly control was obtained when a nonformulated spore powder obtained from Bioferm Corporation was fed at rates up to 142×10^6 spores per gram of feed. No data were taken on feed consumption in this test but palatability of the feed did not seem to be affected.

Because of the variation in results obtained using equivalent dosages from different sources, and between workers, there is a need for standardization and some method of assaying formulations for toxicity.

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Fate of Apple Maggot and Plum Curculio Larvae in Apple Fruits Held in Controlled Atmosphere Storage[1]

E. H. GLASS and P. J. CHAPMAN, *New York State Agricultural Experiment Station, Geneva*

AND

R. M. SMOCK, *Cornell Agricultural Experiment Station, Ithaca, N. Y.*[2]

ABSTRACT

Controlled atmosphere (CA) storage which involves 32° to 38° F. temperatures, oxygen concentrations of 3% and carbon dioxide levels of 2% to 8% is being used to prolong storage and shelf life of apples. The survival of larvae of the apple maggot (*Rhagoletis pomonella* (Walsh)) and plum curculio (*Conotrachelus nenuphar* (Herbst)) in apples held in this type of storage as compared with standard cold storage at 32° F. was studied using naturally infested Twenty-Ounce and Northern Spy apples and thinning apples artificially infested with plum curculio. Results show standard storage was lethal to apple maggot and plum curculio larvae in 33 days or less whereas CA storage at 38° F. was lethal in less than 60 days. Plum curculio larvae were unable to survive 38° F. CA storage for 33 days. The results show that the minimum storage time (90 days) required for certified controlled atmosphere apples is completely lethal to any apple maggot and plum curculio that might be present in the fruit.

The storing of apple fruits under CA (controlled atmospheric) conditions has become an increasingly popular practice in northeastern United States (Smock 1958). This method enables a grower to keep short storage life varieties like McIntosh in prime condition until late spring or early summer. And since high quality apples are often in short supply at this season CA stored fruit often commands good prices.

While eastern CA stored apples have been sold widely over the United States, it has not been possible, until quite recently, to fulfill a demand for this produce in California. The obstacle was a plant quarantine regulation which denied entry to the State of fresh eastern apples of any description. Instituted in 1925, the regulation was first directed against the oriental fruit moth, *Grapholitha molesta* (Busck). Later it was extended to include the plum curculio, *Conotrachelus nenuphar* (Herbst) and the apple-blueberry maggot, *Rhagoletis pomonella* (Walsh).

On April 12, 1960 the California Department of Agriculture issued (Anon. 1960) an amendment to their "Plum Curculio and Apple-Blueberry Maggot Exterior Quarantine" regulation which permitted entry of properly stored eastern CA apples to the State. What influenced officials to make this change was evidence obtained by the writers showing that neither plum curculio nor apple maggot larvae were able to survive the 90-day minimum holding prescribed for CA storage. The present paper consists of a report on these studies together with some relevant background information.

Of the three species covered in the California regulation both the apple maggot and plum curculio are native to eastern North America. They are not known to occur in California, or for that matter, elsewhere in the world outside of what is believed to have been their original distribution range in nature. Currently, the apple maggot may be found as far south as Georgia (in the mountains) and as far west as the Dakotas. It is most consistently troublesome as an apple pest in New York and the New England States, and in the eastern provinces of Canada. The plum curculio occurs throughout Eastern United States and in the adjoining provinces of Canada (Chapman 1938). *C. nenuphar* is two-brooded in the approximately southern half of its range. In the north, and where practically all of the commercial apple crop is grown, it is single brooded. For practical purposes the apple maggot may be considered a single brooded species.

Now in regard to the possibility of these two species being introduced to a new district through the shipment of infested or injured fruit, the chances are much greater for this to occur in the case of the apple maggot than for

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[2] This study was conducted in cooperation with Entomology Research Division, U. S. Department of Agriculture. It was undertaken at the request of Dr. B. A. Porter, Chief of the Fruit and Vegetable Insects Research Branch.

the plum curculio. This is evident first from the fact that larvae of the former species complete their development in mature fruits. In the latter, this activity is confined, for practical purposes, to the immature fruits which drop to the ground in mid-summer. The relevant particulars of these two situations will be discussed separately.

Apple maggot adults will deposit their eggs in the fruits of any variety. The ability of larvae arising from these eggs to complete development, however, varies greatly between apple varieties. There is a close relationship between survival rate and ripening date for the variety. This is to say, the larvae find conditions most favorable for development in the early varieties, poorest in the winter sorts. Chapman (1933) has reported on the influence of variety on larval survival. Expressed as 100-fruit samples he obtained these records on lots which showed a similar high degree of initial infestation. Wealthy, 823 larvae; Twenty-Ounce, 699; McIntosh, 615; Rhode Island Greening, 231; Red Delicious, 15; Ben Davis, 0 from one lot, and 1 from a 216-fruit sample. It should be appreciated that the survival rates just given are probably higher than would occur in lightly infested commercial lots because these heavily infested fruits matured from 1 to 4 weeks ahead of uninfested fruit, thus favoring survival.

In regard to the question of how long larvae may persist in harvested fruit, this again is largely determined by variety. Another variable is the temperature at which the fruit is held after harvest. The larvae make little progress through the flesh of apple until it softens and is past good eating quality by man's standards. Perhaps one could say that for varieties picked between September 15 and October 15 the interval between harvest and first larval emergence under outdoor temperatures would be about 3 to 4 weeks. Chapman (1933) has provided a composite record on larval emergence from 20 varieties held under outdoor conditions. Represented in this series were lots which ranged in picking date from late July to late October. Although most of the larvae were found to emerge from the fruit during August and September emergence continued on into early December.

Some plum curculio larvae have been reported (Chapman 1938) to develop successfully in mature apple fruit. Such occurrences, however, are of more academic than practical interest. For under New York conditions anyway, these records have essentially been limited: to heavily infested fruit in abandoned orchards, to early maturing varieties, and to lots which because of infestation, matured one or more weeks ahead of the normal time for this variety. In New York, plum curculio larvae develop almost exclusively in the imperfectly fertilized apple fruits which drop to the ground in late June and July or the so called June drops. The chances of larvae occurring in commercial lots of varieties ripening with McIntosh and later are virtually nil.

The apple maggot was first subject to regulatory action in 1930. At that time European countries under the leadership of the United Kingdom decided to put an end to the flooding of their markets with so much low grade American fruit. Part of the action taken was to deny admittance to apples which showed any degree of infestation by the apple maggot. In an attempt to meet the general and specific objections made to some American fruit, the Congress in 1933 passed the so-called Export Apple and Pear Act (Anon. 1959). This law established minimum grade and other standards for export fruit and did much to alleviate the problem.

In view of the specific action taken against the apple maggot, interest developed in having some practicable method of freeing the fruit of any live eggs or larvae that might be present. Chapman (1933) and Chapman & Hess (1941) have shown that this can be done readily, by holding infested fruit for about a month under standard cold storage temperature at 32° ± 1°. They also established that all larvae would be killed within about 45 days at 36° F. At 40° F., however, about 1% of the larvae survived. Based on the foregoing studies Federal regulatory officials elected to prescribe refrigeration as a means of qualifying slightly infested fruit for export under the Export Apple and Pear Act. The provision adopted called for a minimum holding period of 40 days at 32° F.

In view of the foregoing, when interest developed recently in marketing eastern CA stored apples in California the question inevitably was raised as to the ability of apple maggot and plum curculio larvae to survive the minimum holding period of 90 days maintained for CA stored fruit. Studies were initiated by the writers in 1959 to determine whether this was true.

Answers were sought to these questions: how susceptible is the plum curculio larva to refrigeration temperatures under both standard and CA conditions; to what degree, if any, is susceptibility of the two species influenced by CA conditions; and finally can the larvae survive the maximum temperature used for holding some varieties of fruit under CA conditons, namely 38° F.

CONTROLLED ATMOSPHERE STORAGE.—The controlled atmosphere storage practice has been described by Smock (1958). A comparison between the gas content of the atmosphere in standard and CA storage is given in table 1. The gas concentrations in standard storage vary little from normal air. In CA storage, however, the carbon dioxide content is raised to and maintained at from 2% to 8% (depending upon variety) while the oxygen content is reduced from 21% to 3%. The temperatures required vary with the variety but the maximum for this type of storage is 38° F.

By law in New York, strict requirements must be met

Table 1.—Atmospheric and temperature conditions for standard 32° and controlled atmosphere storage.

TYPE STORAGE	TEMPERATURE °F.	PER CENT	
		Carbon Dioxide	Oxygen
Standard	32	0.03–1	20–21
Controlled-Atmosphere			
McIntosh	38	5[a]	3
Delicious	32	2–3	3
Golden Delicious	32	2–3	3
Rome Beauty	32	2–3	3
Northern Spy	38	8	3
Stayman Winesap	32	5	3
Baldwin	32	2–3	3
Jonathan	32	3	3
Macoun	38	5	3

[a] For the first month it was 2–3.

if the fruit is to be sold as CA stored apples. These conditions include a 90-day minimum storage period under the conditions given in table 1.

MATERIALS AND METHODS.—In New York apple maggot flies lay eggs from late June to early September (Glass 1960). The largest numbers are laid in July and early August. The maggots leave the fruits in largest numbers in late August and early September (Chapman 1933). In selecting fruit for the present study it was necessary to pick it in August to insure adequate numbers of larvae and suitable condition of the fruit for storage. The fruit selected for the principal part of the tests was the variety Twenty-Ounce and it was picked August 18. We were obliged to hold the test fruit in cool storage at 45° F. until September 25 which was the earliest date a CA storage room was available for sealing. These fruits averaged 18 apple maggot egg punctures per apple and 0.9 plum curculio egg laying scars. Apples of the Northern Spy variety were also included in the test. This fruit was picked on September 10 and was stored at 45° F. until the CA room was ready. The Spy fruit was not so heavily infested with apple maggot as the Twenty-Ounce fruits but bore almost as many plum curculio egg laying scars. Since as already noted (Chapman 1938) plum curculio larvae do not normally occur in picked apples it was necessary to artificially infest some, using stored thinning apples. The technique used was that described by Smith (1957). The infestation schedule was so timed that the developing larvae were about half grown on September 10 when they were placed in the 45° storage along with the Northern Spy apples infested with apple maggot.

On September 25 half of the apples of each variety were placed in standard 32° F. storage and half in a CA storage room which was maintained for conditions required for the McIntosh variety at 38° F. The crates were so arranged that one crate of Twenty-Ounce apples could be removed from each storage room at monthly intervals during the subsequent 6 months. In addition 10 crates of each variety were placed in each storage room for removal when the CA room was scheduled to be opened in April. Packages of 50 curculio-infested thinning apples were placed in each of the Twenty-Ounce crates scheduled for removal at monthly intervals.

The standard storage room averaged 32° F. and ranged from 31° to 33° F. The relative humidity here was 90% to 95% throughout the storage period.

The CA storage room was maintained for McIntosh, the variety most often held in this type of storage. The oxygen level dropped to 5% in 20 days and to 3% in 25 days. Throughout the remainder of the storage period the oxygen concentration ranged from 2.4% to 3.4% with an average daily reading of 3%. Carbon dioxide reached 5% 4 days after sealing and fluctuated between 4.7% and 5.5%. The temperature ranged between 37° and 38° F. The relative humidity fluctuated from 90% when the refrigerant was being pumped through the cooling coils and 98% when it was not. Both the CA and standard storage rooms were of equal size and with equivalent air distribution.

Survival of apple maggot and plum curculio was determined by placing the fruits on ¼-inch-mesh wire racks suspended over pans of water. Mature maggot and curculio larvae left the fruits and dropped to the water where they were collected and counted at regular intervals. Fruits were held on racks until such time as all larvae present had emerged.

Table 2.—Survival of apple maggot in apples held in CA or standard 32° F. storage.

NUMBER OF CRATES	DAYS STORAGE		APPLE MAGGOT LARVAE EMERGING PER CRATE	
	45° F.	32° or CA	32° F.	CA at 38°
		Twenty-Ounce		
2	0	0	1,324	1,324
2	36	0	506	506
1	36	33	0	3
1	36	60	0	0
1	36	88	0	0
6	36	132	0	0
6	36	179	0	0
		Northern Spy		
1	0	0	101	101
1	15	0	74	74
5	15	132	0	0
5	15	179	0	0

RESULTS.—Detailed data on survival of apple maggot larvae from apples held in standard 32° F. and CA storage for various intervals are given in table 2. The records show that in the Twenty-Ounce control lots 1,324 larvae were recovered from fruit that was not subject to any kind of cool or cold storage and 506 larvae from the lot held 36 days at 45° F. It is possible that some maggots matured and left the fruit during this 45° F. preliminary holding period. In the Northern Spy controls only 101 larvae per crate were recovered from the apples which were not subjected to any storage and 74 per crate in the lot held 15 days at 45° F.

No plum curculio larvae were recovered from any of the field-collected apples whether stored or not.

The data in table 2 for the standard 32° F. storage show complete mortality after 33 or more days in storage. These confirm the results obtained earlier by Chapman & Hess (1941). In CA storage there were three survivors (0.6%) at 33 days but none after 60 or more days. These records are based on a calculated presence of 8,330 live maggots in the samples when placed in each of the storages on September 25.

The results for plum curculio larvae in the thinning apples are given in table 3. The sample of 50 apples not cool stored produced 232 mature larvae and the one held for 15 days at 45° F. yielded 147. None survived 33 days

Table 3.—Survival of plum curculio larvae in thinning apples held in CA or standard 32° F. storage.

DAYS AT 45° F.	DAYS IN CA OR 32° STORAGE	NUMBER SURVIVING	
		32°	CA at 38° F.
0	0	232	232
15	0	147	147
15	33	0	0
15	60	0	0
15	88	0	0
15	132	0	0
15	179	0	0

standard storage at 32° F. or 38° F. under CA conditions. Dead larvae were found within these fruits.

The only other insect found to be present in the field-collected apples was the codling moth, *Carpocapsa pomonella* (L.). There were too few to make valid comparisons between the survival rates in the two types of storage. It was observed, however, that some codling moth larvae did survive 132 days holding in both CA and standard 32° F. storage.

DISCUSSION.—The results show quite clearly that CA storage as used in this study is lethal to both apple maggot and plum curculio larvae. The lethal action of CA storage was comparable to that for standard cold storage when the temperature differential is taken into account. It is apparent that temperature of storage is either the sole or at least the controlling operational cause of mortality. There is no evidence from this study that the reduced oxygen and increased carbon dioxide concentrations measurably delayed or hastened mortality of apple maggot and plum curculio larvae. CA storage was not lethal to cold resistant codling moth over a 132-day period. Perhaps this is not too surprising since this species overwinters in the larval stage. The experiments reported herein were not designed to determine critically whether the modified atmosphere in CA storage had any favorable or unfavorable influence on the insects in question. But the results do show there was no practically significant effect exerted. It therefore follows that any minor variations in storage air conditions should not be of any importance regarding insect survival provided the temperature is maintained at 38° F. or less.

Although plum curculio larvae are not normally found in picked fruit in New York the possibility of this occurring in apples grown in the two-brooded zone of the Southern States cannot be totally disregarded. Even if larvae might theoretically occur in picked fruit the data presented here show that they would not survive even the minimum CA storage period of 90 days.

CONCLUSIONS.—CA storage conditions involving temperatures of 38° F. or less are 100% lethal to plum curculio larvae in 33 days and probably to apple maggot larvae in considerably less than 60 days. Thus apple fruit held in CA storage for the 90-day minimum length of time required for certification would be completely disinfested of live apple maggot and plum curculio.

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Pear Leaf Scorch and Its Relation to the European Red Mite[1]

G. G. DUSTAN and A. B. STEVENSON

ABSTRACT

Observations and experiments from 1958 to 1960 showed that the characteristic scorching of pear foliage that occurred during hot weather in late July and early August in the Niagara Peninsula, Ontario was directly associated with the incidence of European red mite (*Panonychus ulmi* (Koch)) and could be largely prevented by controlling the mites. Less than three mites per leaf in July sometimes resulted in leaf scorch on Bartlett, Bosc, and Anjou varieties. The Kieffer variety was more resistant and more mites were needed to produce scorching.

Blackening, or scorching, of pear foliage has been observed for a number of years in the Niagara Peninsula, Ontario, particularly during periods of high temperature in late July or early August. It was of minor importance until 1958 when it became severe in many orchards. The injury was in the form of blackening of entire leaves or parts of leaves and was generally more severe in the mid-central parts of the trees. Scorched leaves often occurred in clusters on a shoot. The affected leaves usually fell prematurely. Scorching occurred most frequently on Bartlett, Bosc, and Anjou varieties but only in two seasons was it observed on the Kieffer variety. The injury was similar to that occurring in New York State where Parker (1958) described it as pear leaf scorch and considered high temperature to be the primary cause.

During 1958 it was noted that although the scorching resembled the injury sometimes caused by parathion during hot weather it occurred in some orchards where this insecticide had not been used, and in others not until 2 or 3 weeks after its application. Observations of affected orchards gave some evidence of the importance of temperature and soil moisture relationships to the scorching but, in addition, there was a consistent association between the degree of injury and the incidence of the European red mite, *Panonychus ulmi* (Koch).

Newcomer (1933) described browning and drying of the leaves of certain varieties of pears in the Pacific Northwest of the United States in hot weather, and stated that the condition appeared to be aggravated by severe infestations of mites. Madsen & Borden (1955) reported that in

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California a population of from four to five mites per leaf could result in bronzing and burning of pear foliage and Madsen & Westigard (1960) stated that burning was especially severe when temperatures of 90° F. or over were reached. Cant (1960) showed that a leaf scorch of pear in Australia was associated with the two-spotted spider mite, *Tetranychus telarius* (L.), and that severe scorch could follow a heat wave even if the mite population was only two or three per leaf.

This paper presents the results of observations and experiments which show that the European red mite is probably the principal factor in determining the occurrence of pear leaf scorch in the Niagara Peninsula, Ontario.

1958 RESULTS.—When pear leaf scorch became widespread a survey was made, August 11 to 14, 1958, in 28 orchards, chiefly of the Bartlett variety. Samples of 25 leaves (2 per tree) were taken from each orchard and the numbers of active stages of the European red mite counted. The leaves were chosen as randomly as possible, except that badly scorched leaves were not taken because the mites might have left them. In 9 orchards showing moderate to severe leaf scorch the numbers of active mites per leaf ranged from 1.3 to 15.4, with an average of 5.5. In 11 orchards, similar in size and growing conditions, but showing little or no leaf scorch, the numbers of active mites per leaf varied from 0 to 4.8 with an average of 0.8. Thus, mites were approximately seven times more numerous where leaf scorch was common than in unscorched orchards.

Moderate leaf scorch and a population of 4.6 mites per leaf were observed in the north end of one orchard, whereas scorching was absent from the remainder of the same orchard where mites averaged less than one per leaf. The association of mites and leaf scorch was shown in another orchard, where leaves taken from branches having many scorched leaves averaged 4.4 mites per leaf whereas leaves from unaffected branches on the same trees averaged only 0.5 per leaf.

The importance of varietal susceptibility was seen in two pear orchards of the Kieffer variety where there were averages of 21.2 and 5.0 active mites per leaf. There was no scorching of the Kieffer foliage in these orchards, though typical bronzing of the foliage from mite feeding was evident. However, the foliage of other varieties, Bartlett and Bosc, that had been grafted onto the Kieffer trees to serve as pollinators, was heavily scorched.

In general, young Bartlett pear orchards showed very little leaf scorch whereas young trees interplanted in old orchards were usually more severely scorched than the older trees. Also, in some cases, scorching was noticeably less on the outside rows than further into the older Bartlett pear orchards. It is not known whether these differences in scorching were due to differences in mite abundance or heat and soil moisture relations in the two locations.

1959 RESULTS.—In 1959, a hot, dry season, scorch became evident in mid-July, about 10 days earlier than in 1958, and both European red mite and scorch were more prevalent.

Observations and leaf collections made in 11 Bartlett pear orchards on August 5 showed that the average numbers of active stages and of eggs of mites per leaf in 8 orchards with moderate to heavy scorch was 15.9 and 74.9, respectively, whereas in 3 orchards showing little or no scorch there were averages of only 3.8 active stages and 31.3 eggs per leaf.

In a Bartlett pear orchard at Winona, Ontario, 5 rows were sprayed on July 2 with 25% malathion, 2 lb. and 50% ovex, 0.5 lb. per 100 gallons, and 5 adjoining rows with malathion alone. Counts on July 6 showed 5 active stages of the European red mite per 100 leaves where ovex was used and 332 in the malathion plot. Leaf scorch had appeared in the orchard by the end of July. A heavy rain on July 29 knocked off many of the scorched leaves and these were counted under 13 trees in each plot. There were from 6 to 30, with an average of 15 fallen leaves per tree where mites were controlled by ovex, and 34 to 190, with an average of 111 fallen leaves, where ovex was not applied.

1960 RESULTS.—The first half of the 1960 growing season was cool, and late, and scorch developed later than usual. Mites were very scarce until late July and then built up rapidly. Leaf scorch started to appear in a number of pear orchards during the last week in July and was first observed on the side of a row of Bartlett trees adjoining a heavily infested peach orchard. The scorched sides of these pear trees averaged 13.5 mites per leaf compared with 5.6 on the sides with little or no scorch.

In a Bartlett orchard at Queenston, Ontario, that had been severely scorched in 1959, the grower used Tedion® (p-chlorophenyl 2,4,5-trichlorophenyl sulfone) with malathion in the calyx spray in one-half of the orchard and omitted the Tedion in the other half in 1960. On August 5 both scorch and mites were almost entirely absent where Tedion had been used, whereas in the other half of the orchard mites averaged 22.8 per leaf, and leaf scorch was severe.

In part of another orchard, at Port Dalhousie, Ontario, ethion was substituted for parathion-DDT in the three regular pear sprays in May, July and August. On August 31, there were 16 mites per leaf and severe leaf scorch where parathion and DDT were used, and 0.8 mites per leaf and only a trace of scorch on the ethion-sprayed trees.

Records in two Bartlett pear orchards in early August showed that scorching developed earlier and was more severe on the parts of the trees where mites were most plentiful. In the first orchard the west sides of the trees were moderately to severely scorched and had an average of 39.4 mites per leaf. The east sides of the same trees were relatively lightly scorched and had only 11.8 mites per leaf. In the second orchard, severely scorched areas of the trees averaged 38.1 mites per leaf and lightly scorched areas 6.2 mites per leaf. The differences in the degree of scorching between the heavily and lightly mite-infested area was much less evident by the middle of August.

In 1960, scorch was observed in a few orchards of the Kieffer variety for the first time in several years. In one Kieffer orchard where scorch and leaf drop were especially severe, there were 27.7 mites per leaf in early September. However, it was observed during the surveys in 1958 and 1959 that far more mites are needed to initiate scorching of Kieffer foliage than that of Bartlett pear.

An experiment was conducted in 1960 in a Bartlett pear orchard at Port Dalhousie, Ontario, to demonstrate the

Table 1.—Pear leaf scorch and European red mite abundance on Bartlett pear. Port Dalhousie, Ontario, 1960.

Acaricide Treatments[a]	Amounts per 100 Gal.[b]	Average Scorch Rating per Tree		Average Number of Mites per 100 Leaves	
		Aug. 11	Sept. 1	Aug. 11	Sept. 1
Kelthane®[c] 18.5%, WP in cover sprays	1.5 lb.	0.3	0.9	1.3	0
Guthion®[d] 25%, WP in calyx and cover sprays	1.25 lb.	0.5	0.6	0	5.3
Kelthane 18.5%, WP in calyx spray	1.5 lb.	2.4	2.5	16	355
Ethion-oil,[e] E in dormant spray	2 gal.	2.7	2.7	171	945
Dormant oil,[f] E in dormant spray	3 gal.	4.0	4.1	457	2579
Check, no acaricide		3.4	3.9	1035	3186

[a] Dormant spray, April 13; calyx spray, May 27; cover sprays, July 7 and August 1. All treatments except Guthion, but including the check, included 15% parathion, 1 lb. per 100 gal. in the calyx and cover sprays, lead arsenate 4 lb. in the calyx spray, and 50% DDT, 2 lb. in the cover sprays. Ferbam, 2 lb. was added to the calyx spray in all treatments and the check.

[b] Imperial gallons.

[c] 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol.

[d] *O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phophorodithioate.

[e] 2% ethion and 95% emulsible oil (Niagara Brand Chemicals).

[f] Shell Neutrol (Visc. 200–215).

relation between the European red mite and leaf scorch. The treatments (table 1) were replicated three times and each plot contained five trees in a row. Individual trees were rated as to degree of injury on August 11, and again on September 1 by the same observer, using a rating system with the following categories:

0—No scorch.
1—Trace. 2–10 scorched leaves per tree.
2—Light. 1–10 scorched clusters of 4–10 leaves each per tree.
3—Moderate. 1–10 scorched clusters of 11–25 leaves each per tree.
4—Moderately severe. Several large scorched areas of 25 or more leaves each per tree.
5—Severe. Large sections of one-tenth to one-quarter or more of the foliage scorched.

On the same days, samples of 25 leaves per plot (5 per tree) were taken and the numbers of active stages of the European red mite counted. As in all previous samples, severely scorched leaves were not taken but leaves were often taken near scorched clusters of leaves. Table 1 shows the results of this experiment which showed that light scorch appeared on trees where the mite population was only 0.16 per leaf on August 11 and that it was more severe in the plots where mites were more abundant. It is interesting to note that scorch on August 11 was just as severe in the plots with 4.57 mites per leaf as in the ones with 10.35 per leaf, and that there was little increase in scorching between August 11 and September 1, though the mite population increased about three to five times. In general, this appeared to be the case in other orchards with scorching reaching nearly its maximum by the middle of August.

No attempt was made to estimate the effect of leaf scorch on the size of the crop or growth of the trees. From general observations it appeared that, even on the most seriously scorched trees, less than one quarter of the leaves were appreciably affected. Although the scorching and premature dropping of the leaves must have been harmful, it was noted that some Bartlett pear trees set and matured good crops following two seasons of moderate to severe leaf scorch.

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Utilization of a Nuclear Polyhedrosis Virus to Control the Cabbage Looper on Cole Crops in Virginia[1]

R. N. Hofmaster, *Virginia Truck Experiment Station, Eastern Shore Branch, Painter,*
and L. P. Ditman,[2] *University of Maryland, College Park*

Abstract

Large-scale field experiments on the Eastern Shore of Virginia during 1960 indicated that a nuclear polyhedrosis virus could be artificially disseminated either alone or in insecticide sprays to commercially control the cabbage looper, *Trichoplusia ni* (Hübner). Weekly applications of virus in the insecticide sprays in commercial plantings maintained the looper populations at an extremely low level throughout the growing season. Intensities of looper population resulting from natural virus infections on untreated broccoli at Painter, Virginia, demonstrated a cyclic behavior that was not observed at College Park, Maryland. At College Park the nuclear polyhedrosis virus is believed to have naturally occurred early in the season while looper populations were at a low level and the population remained low throughout the season, June through October. These observations indicate that frequency of treatment by the virus for commercial control may vary in different areas.

Following the second World War, the cabbage looper, *Trichoplusia ni* (Hübner), has become increasingly resistant to insecticides in southeastern Virginia. DDT, the pyrethrins, toxaphene, endrin, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) and other chemicals have all declined in effectiveness to such an extent that satisfactory commercial control has been almost impossible to attain. Results from insecticides have been especially poor during hot, dry periods.

Since 1955, heavy local infestations of cabbage loopers in southeastern Virginia have been brought under control by a disease which has been identified as a nuclear polyhedrosis virus. This is apparently the same disease reported in Virginia by Walker & Anderson (1936) who stated that "Outbreaks of cabbage loopers are frequently almost completely destroyed by disease that causes their bodies to turn pale yellow and rot."

Semel (1956) commented on the efficiency of a nuclear polyhedrosis virus in reducing looper populations in Long Island and has also summarized a history of the incidence of looper polyhedrosis throughout the country. Hall (1957) and McEwen & Hervey (1958–1959) conducted limited field tests on small plots with a polyhedrosis on loopers in California and New York, respectively, and believe that the virus could be disseminated effectively in the field. Preliminary observations in Virginia, 1956–59, also indicated the possibility of field use of the virus as a control measure.

During the summer of 1960 a cooperative virus propagation and field scale application program was initiated by the entomology departments of the University of Maryland and the Virginia Truck Experiment Station.

Materials and Methods.—Cabbage loopers were reared at the University of Maryland at College Park. A small plot of broccoli was planted in early spring as a source of rearing material and for food for the larvae in the laboratory. The first looper larvae appeared in late June, were collected and reared to adults from which eggs were obtained for mass production of larvae in the laboratory. The general procedure suggested by McEwen & Hervey (1960) was followed. When nearly grown, the loopers were transferred to the Virginia Truck Experiment Station and infected with the nuclear polyhedrosis virus as described below.

Broccoli leaves were dipped in a virus suspension prepared at the rate of five thoroughly macerated virus-infected loopers per 100 gallons of water. These treated broccoli leaves were then placed in waxed gallon ice cream containers fitted with wire gauze tops, and 200 larvae added. The loopers were checked for disease twice daily, removed when obviously infected, counted and placed in a glass beaker for 48 hours. Best results were obtained by removing the loopers from the rearing cartons as soon as the characteristic yellowish white color appeared and they had become more or less quiescent. If the larvae were not removed before death occurred it was almost impossible to handle them, as they disintegrated completely into "mush." At the end of the 48-hour period the infected loopers were either placed in the deep freeze intact at 0° F. or macerated in a Waring Blendor to which distilled water at the rate of 1 cc. per looper had been added. This stock solution was held in the freezer until needed.

A regular series of field applications of the virus suspension was made on approximately 150 acres of broccoli, kale and collards in the Keller-Cape Charles, Virginia area. Entire fields or sections of at least 20 acres were sprayed. Treatments were made at weekly intervals with high pressure boom-type sprayers covering 12 to 14 rows and delivering 75 to 100 gallons of water per acre at a pressure of 300 p.s.i. The broccoli received a virus suspension containing the equivalent of five infected fifth-instar loopers per acre; however, owing to the increased difficulty in coverage, the rate was increased to 10 infected loopers per acre in the kale and collards. Following the first treatment at Keller and the second treatment at Cape Charles, the virus was combined with several insecticides. The polyhedra count in the virus suspensions was not determined.

The efficiency of the virus in controlling the looper was evaluated by taking weekly or near weekly field counts from August 16 through October 26. One hundred plants from each of four representative sections of a field were examined for larvae at each sampling date.

Results.—In table 1 results of counts of cabbage looper larvae in broccoli fields receiving regular nuclear polyhedrosis virus treatments at Keller and Cape Charles,

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[2] The authors appreciate guidance and encouragement given by Dr. F. L. McEwen and Dr. Maurie Semel.

Table 1.—Mean number of cabbage loopers per 100 broccoli plants after weekly applications of a nuclear polyhedrosis virus at the rate of five diseased fifth-instar larvae per acre, Eastern Shore of Virginia.

Sampling Date (1960)	Mean Number of Loopers on Indicated Sampling Date[a]		
	Keller	Cape Charles	Untreated (Painter)
8/16	437	—	—
8/24	1	1,612	1,361
9/8	1	20	453
9/13	0	1	30[b]
9/20	0	0	212
9/28	1	0	1,165
10/5	0	1	1,022
10/11	1	1	563
10/19	0	0	118[b]
10/26	0	0	20

[a] Mean of 4 replicates.
[b] Looper populations decreased due to natural infection by the virus.

Virginia are compared with those made in untreated broccoli at the Experiment Station at Painter. One fact is readily apparent: looper populations dropped to a low level during the week following the initial virus applications at both Keller and Cape Charles and this low level was maintained throughout the remainder of the season. This is in direct contrast to the untreated broccoli where considerable fluctuation in looper populations occurred despite the heavy incidence of a natural virus infection.

The performance of the virus in the Cape Charles area was especially striking. In the 2 weeks following the initial application of virus the number of loopers was reduced from 1,602 per 100 broccoli plants to a low of only 20 per 100 plants—a decrease of more than 98%. Many sick loopers showing typical virus symptoms were observed in the field during this time. That this condition was due to the virus and not insecticide residues from an endrin-parathion treatment applied 3 weeks previously was clearly indicated when healthy loopers, fed broccoli leaves dipped in suspensions prepared from these affected loopers, developed identical symptoms. The role of the virus sprays in disseminating the virus was further substantiated by the fact that untreated broccoli adjacent to the virus-treated plots had little or no natural nuclear polyhedrosis infection at this date.

Part of the broccoli at Keller received the initial virus application in conjunction with an endrin-parathion spray while the remainder of the field was treated only with the virus. Both treatments were equally effective, further demonstrating the efficiency of the virus and also that insecticide-virus combinations are feasible.

The data obtained from these tests show that regular applications of the virus were more effective than natural infections. It is noteworthy that untreated broccoli was heavily reinfested with loopers even though a naturally occurring nuclear polyhedrosis had killed most of the original looper population. As the number of loopers increased again the virus eventually brought the infestation under control, but not before serious damage resulted.

The first nuclear polyhedrosis sprays were applied to kale and collards at Painter while the plants were still small and before an appreciable number of loopers was present. Subsequent regular weekly applications of virus held the loopers in check throughout the season with a peak of only two loopers per 100 plants being recorded.

Discussion.—Cabbage loopers have become so resistant to insecticides that, in order to produce broccoli acceptable for processing, growers on the Eastern Shore of Virginia have been forced to adopt a 5- to 7-day treatment schedule throughout the season. In such a program the cost of insecticides alone may surpass $50 per acre and yet provide no definite assurance of acceptable broccoli. Looper control in kale, collards and salads is even more difficult because some of the most promising chemicals have not been cleared for use on these groups.

The data presented in this study strongly suggest that large-scale field applications of a nuclear polyhedrosis virus could be used for control of the cabbage looper. It was generally conceded by entomologists and the personnel of food packers concerned that better looper control was obtained in virus-treated broccoli in 1960 than at any other time during the past 10 years. Likewise, for the first time in years, it was possible to harvest kale and collards for processing with reasonable assurance that the finished pack would be acceptable.

The number of applications of the virus or the polyhedra content necessary to hold a looper population in check was not established. Contrary to what was anticipated, in Virginia heavy cabbage looper populations were able to develop on new growth in a 2- to 3-week period following almost complete elimination by a natural occurrence of polyhedrosis. In view of this fact, presumably, more or less regular applications of virus at 2- to 3-week intervals may be necessary, although the frequency will probably depend to a large extent upon the potency of the virus, number of loopers present, growth stage of the plant, and general weather conditions. Provision of an early source of inoculum by treating in the plant bed or while the plants are still small may decrease the number of sprays required.

The cyclic behavior of looper populations under natural occurring virus infection was not observed at College Park in 1960. In 6 years, 1954 to 1959, of insecticidal work with broccoli on the Plant Research Farm at College Park, the second author cannot remember seeing a single looper larva in the field that was infected with nuclear polyhedrosis virus. There is strong evidence that in 1960 the disease apparently occurred naturally. The first larvae collected in the field, and fed field foliage, matured normally. By the time the second brood had nearly reached maturity virus-infected larvae appeared and the third brood larvae were completely killed, apparently entirely by polyhedrosis virus present on the food brought in from the field. Since the larvae were reared in a new laboratory in new equipment and since the first generation of larvae matured with no incidence of disease the assumption that the virus was present in the field is probably correct. Observations on the seasonal looper population at College Park are therefore worthy of note. The early planting of broccoli was followed by two fall plantings; one by the Horticulture Department received weekly sprayings of malathion, the other for residue work received a treatment with phosphamidon and contained untreated areas. The looper population remained low throughout the season, and never exhibited the cyclic

peaks experienced in the population on the Experimental Farm at Painter, Virginia. Complete control of all insects was obtained at College Park on the broccoli treated with malathion. Several field examinations and examination of the harvested crop revealed no aphids, imported cabbageworm (*Pieris rapae* (L.)) or cabbage looper. The untreated areas of the planting made for insecticide residue studies appeared to be free from cabbage looper during the entire growing season, though some imported cabbageworm larvae were present. In reviewing the data taken on the broccoli experiments at College Park of the past 7 years it was found that light looper populations occurred in 1955, 1958 and 1960 (Ditman et al. 1958, Steinhauer et al. 1959). Though diseased larvae were never observed it is possible that the polyhedrosis virus was a determining factor. Incidentally during seasons of low looper infestation sprays of malathion were highly effective in protecting broccoli from insect injury. If the low seasonal looper population observed at College Park in 1960 was caused by the presence of naturally occurring polyhedrosis virus, then it may be assumed that the effectiveness of applied virus sprays will vary greatly with environmental conditions, and the number and frequency of treatments required for desired results may vary in different areas.

Extensive field observations in Virginia indicate that rainfall apparently aids natural virus distribution but is not essential to virus development. Our most striking examples of control by the natural virus have usually been associated with a period of dry weather, in which heavy looper populations developed, followed by excessive rainfall. Some virus has always been observed in the field during dry periods but generally is of limited effectiveness in controlling the loopers unless the infestation is quite heavy. Spray applications of virus suspensions on the other hand have given excellent control of relatively light looper infestations during dry periods, apparently because of better distribution.

Nuclear polyhedrosis is not active against imported cabbageworms, aphids, corn earworms (*Heliothis zea* (Boddie)), and other insect pests of cole crops. As a result, insecticides for the control of these pests must be included along with the virus. Endrin-parathion, toxaphene-parathion, Phosdrin, Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) and malathion-Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane) were successfully used in conjunction with the virus for control of the entire cole-corp insect complex.

It definitely appears that alternative methods of looper control other than the use of insecticides must be developed if the growing of cole crops is to be continued on the Eastern Shore of Virginia. The use of nuclear polyhedrosis virus offers a possible solution to this problem. As McEwen & Hervey (1959) point out "The ease with which the cabbage looper can be mass produced in the laboratory makes quantitative production of the polyhedrosis relatively easy. There would seem to be no good reason why this insect virus could not be economically produced on a commercial scale."

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Artificial Diets for the Adult Boll Weevil and Techniques for Obtaining Eggs[1]

ERMA S. VANDERZANT and T. B. DAVICH, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., in cooperation with the Texas Agricultural Experiment Station,[2] College Station*

ABSTRACT

The boll weevil (*Anthonomus grandis* Boheman) preferred curved to flat-surfaced diets. On a cylindrically-shaped diet that was hemispheric at one end, the weevils fed and oviposited as well as on cotton buds.

A mechanical method of removing eggs from diet involved breaking up the cylinders with water in a blender, placing the mixture in a sieve that retained the large particles but permitted the small particles and eggs to pass through, and floating the eggs in a sucrose solution to cause further separation.

An effective oviposition diet consisted of enzymatic hydrolyzate of casein, sucrose, salts, cholesterol, B-vitamins, choline, inositol, ascorbic acid, germinated cottonseed, water, and agar. A similar diet with the addition of more ascorbic acid, corn oil, and fat-soluble vitamins was tried. During the first 20 days on these diets, each female laid an average of 114 and 202 eggs, respectively. Since tests were conducted over 12 months and conditions and weevils may have varied, the composition of the diet may not have caused the difference in oviposition. These crude diets are satisfactory for routine rearing.

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Preliminary studies with artificial diets for the adult boll weevil (*Anthonomus grandis* Boheman) were reported by Vanderzant et al. (1959). A basal diet composed of known nutrients and supplemented with various pollens, wheat germ, germinated pea, or germinated cotton seeds supplied nutrients for egg development and stimuli for oviposition. This paper reports improvements in diet and techniques that have made the use of artificial diets satisfactory and economical for routine feeding and oviposition.

EFFECT OF SHAPE OF DIET ON OVIPOSITION.—In previous tests artificial diet in a thin, flat layer in aluminum dishes was used for feeding and oviposition. However, it was observed that weevils responded to round inert objects, such as clay balls, that were similar in shape to cotton squares. Hence, tests were devised to determine adult preference for diet offered with flat surfaces in aluminum dishes and with curved surfaces in cylindrical shapes. A mold (figs. 1 and 2) was devised for shaping the diet into cylinders that were hemispheric at one end. Each mold was constructed from two sheets of plexiglass, the top sheet $\frac{1}{2}$ inch and the bottom sheet $\frac{1}{4}$ inch thick. The sheets were clamped together and holes, $\frac{3}{8}$ inch in diameter, were drilled through the top sheet and $\frac{1}{16}$ inch into the bottom sheet thus making the holes $\frac{9}{16}$ inch deep. Pins were placed at two corners of the mold to insure proper alignment of the holes. Sheet aluminum also was a satisfactory material for making molds. In fact, the diet cooled and solidified more rapidly in aluminum than in plastic molds.

Molten diet was poured into the holes (fig. 1) and allowed to gel; then toothpicks were inserted as handles and the sheets were separated, thus releasing the cylinders (fig. 2). They then were dipped into melted paraffin to prevent drying and stored in a refrigerator until needed.

Table 1.—Effect of shape of diet on oviposition by boll weevils from Mexico on various germinated seed diets.

Diet No.	Shape	Number of Females	Number of Eggs in 20 Days	
			Total	Per Female[a]
1	Flat	6	17	3.4
	Cylinder	7	141	20
	Flat	9	261	34
	Cylinder	5	324	65
2	Flat	7	241	40
	Cylinder	6	291	58
	Flat	8	265	33
	Cylinder	8	291	40
3	Flat	6	267	44
	Cylinder	5	246	82

[a] Corrected for mortality.

One cylinder was used for each six to eight adults (fig. 3). Results of concurrent tests with weevils fed diet in cylinders and dishes showed that adults laid more eggs in the cylinders than similar weevils in the same diet in dishes (table 1). Three different adult diets and weevils reared from two different larval diets were used in these tests. The compositions of the adult diets 1, 2, and 3 (table 1) are not given in this paper because they have no bearing on the problem being discussed.

Adults punctured the wax coating of the cylinders with their beaks and fed and laid eggs in the same manner as in squares. In fact, adults were attracted immediately to

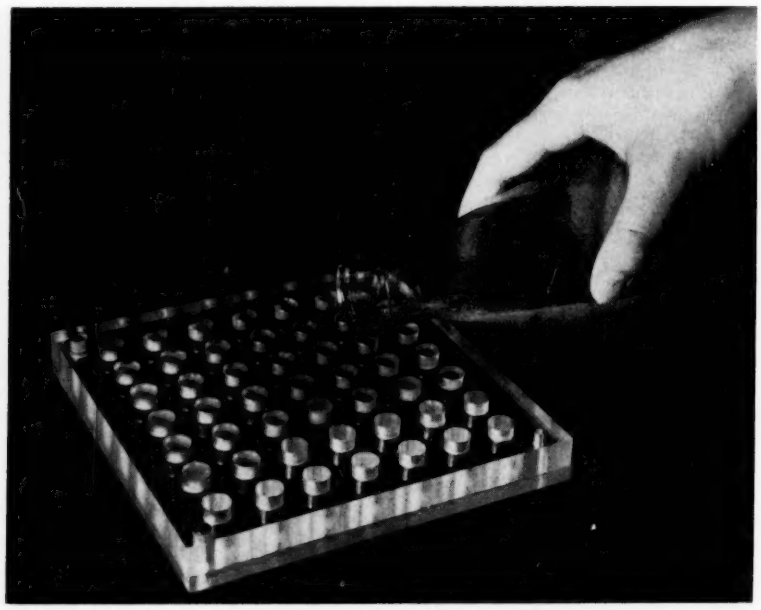

FIG. 1.—Molten diet being poured into a mold.

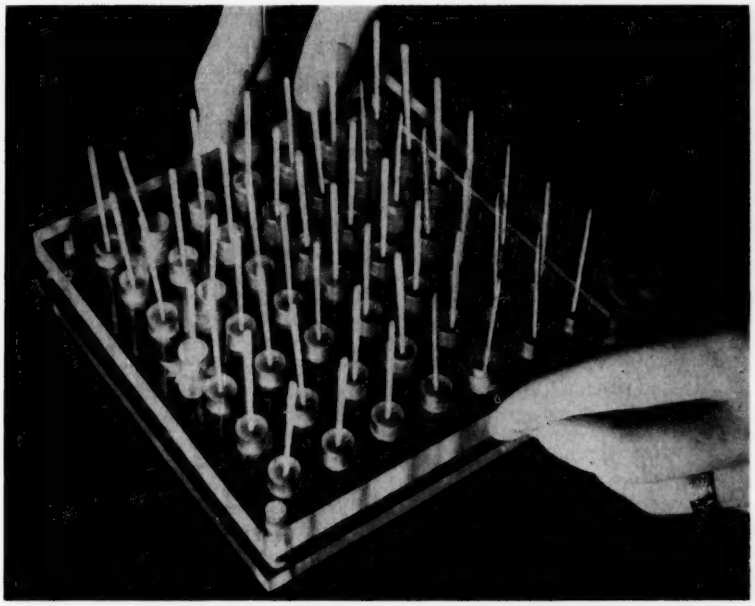

FIG. 2.—Diet cylinders being removed from a mold.

artificial diets when diet cylinders were placed in cages with boll weevils feeding on squares. Even in the presence of squares as many as 71 eggs were laid in a single cylinder. During a 24-hour period females given 10 squares and 2 cylinders laid 50% of the total number of eggs in the cylinders.

Cylinders subsequently were used for routine feeding because their preparation was easier, faster, and more economical than growing, collecting, and preparing squares and because more eggs were obtained per cylinder than per square.

MECHANICAL COLLECTION OF EGGS.—Manual dissection of food material to collect eggs is a tedious process although it is much easier to remove eggs by hand from artificial diet than from squares. Nevertheless, a mechanical method of removing the eggs was desirable to reduce the time and labor involved in rearing. Gast (1961) recently devised a mechanical method of collecting eggs from squares and was able to recover 62% of eggs so laid.

The following process was devised for the mechanical removal and collection of boll weevil eggs from artificial diet cylinders. The paraffin covering was removed from the cylinders by hand. The cylinders then were placed in a Waring Blendor jar containing water at room temperature. Cellophane tape and rubber bands covered the blades on the blade assembly to prevent egg destruction. The blender was run at low speed about 1 minute to break up the cylinders. Then the contents of the blender were washed into a U. S. standard No. 20 sieve (840 micron openings), placed above a U. S. standard No. 45 sieve (350 micron openings), and tap water was run through the sieves. The material left in the No. 20 sieve was submitted to the same treatment in the blender and run through the sieves again. The material collected in the

FIG. 3.—Boll weevils feeding on diet cylinders.

No. 45 sieve from the two blender treatments was transferred with a sucrose solution composed of 28.4 g. of sucrose and 100 ml. of water to a 500-ml. separatory funnel half filled with the same solution. The funnel was allowed to stand about 5 minutes or until the eggs stopped rising to the surface. Two-thirds of the contents of the funnel containing pieces of diet were drained off and the remainder of the solution containing the eggs was transferred quantitatively to a 250-ml. separatory funnel. More sucrose solution was added and the eggs again were allowed to rise to the top. The bottom portion of the solution was removed as before and the eggs were removed from the sucrose solution by pouring them into a funnel fitted with a filter paper. The eggs were rinsed with water, then surface sterilized, and transferred to larval diet as in the usual rearing procedure.

Sixteen cages each with 10 male and 10 female weevils were used to test this method. They were given fresh diet twice daily. Each day for 24 days the diet cylinders from four randomly selected cages were manually dissected whereas those in the other 12 cages were mechanically dissected for eggs. A total of 2,228 eggs were collected by manual dissection and 7,134 eggs by mechanical collection or 55.7 and 59.4 eggs per female, respectively. In the first treatment with the blender 80.7% of the eggs were collected. From the results it appeared that the mechanical method was more effective than the manual process since more eggs per female were recovered and only one-sixth the time was required. There was apparently no damage done to the eggs in the mechanical separation because hatch and survival were the same as for eggs dissected by hand.

COMPOSITION AND PREPARATION OF THE ADULT DIET.—The composition was as follows: 3 g. of enzymatic hydrolyzate of casein, 4 g. of sucrose, 0.75 g. of Wesson's salts, 0.05 g. of cholesterol, 1 ml. of B-vitamin mixture (Vanderzant 1959), 50 mg. of choline, 20 mg. of inositol, 0.2 g. of ascorbic acid, 3 g. of agar, 100 ml. of water, and 10 g. of germinated cottonseed preparation.

The method of processing the cottonseed was as follows: The seed was soaked in water for 2 hours, then placed between wet towels to germinate. The total germination time was 48 to 55 hours. The seed then was squeezed from the hulls and weighed. An equal amount of water and 0.1 g. of ascorbic acid for approximately 75 g. of seed were added and the material was blanched 3 to 5 minutes. It was then beaten to a puree in a Waring Blendor, weighed out in 10-g. portions, and stored in a freezer until needed. Each 10-g. portion represented about 5 g. of dehulled, wet germinated seed.

To prepare the diet all constituents except the ascorbic acid were mixed in a blender, heated in a water bath just long enough to dissolve the agar, then allowed to cool for a few minutes. Ascorbic acid was added just before the diet was poured into the mold.

In some tests lyophilized cotton-square powder, 5 g., was used in place of the germinated seed in the diet described above. To avoid cooking the square powder, it was added with the ascorbic acid in the last step. To prepare the square powder, the bracts and stems were removed and the squares lyophilized. The dried whole squares were ground in a blender, then air-dried for 24 hours, and beaten to a fine powder in the blender. This material was stored in a refrigerator until needed. Acetone-dried square powder also was tried in place of whole-square powder.

Subsequently, in tests with purified diets oviposition appeared to be improved by the presence of fat and/or fat-soluble vitamins in the diet. To correct a possible deficiency of these substances arising from the small amount of germinated seed present in the diet, the following substances were added: 0.5 g. of corn oil, 25 mg. of β-carotene-water-soluble complex (0.6 mg. of β-carotene), 10 mg. of α-tocopherol acetate, 0.5 mg. of vitamin D_3, and 0.5 mg. of menadione. The last three substances were added in ethanolic solution. This diet also contained twice as much ascorbic acid, 0.4 g., as the first one.

Except for important changes in the ascorbic acid and sugar content the germinated seed diets reported above were similar to the one used by Vanderzant et al. (1959) in earlier tests. Weevils laid more eggs when ascorbic acid was present in the adult diet but this vitamin produced the most marked effect upon hatching. Details of the role of ascorbic acid in boll weevil development will be reported elsewhere.

During the tests with different adult diets weevils were found to feed better on a diet containing fructose than diets containing glucose. Several sugars then were tested and the following order of preference was established: galactose, fructose, sucrose, glucose, maltose, lactose, melibiose, and raffinose. The feeding response was greatest with the first three sugars; light feeding occurred with the others. For routine use in crude diets, sucrose was satisfactory. Preference for these sugars, except for galactose, was in the order of sweetness by human taste tests. Galactose obtained from two commercial sources gave similar results.

As in previous studies newly emerged adults, equal numbers of males and females, were put in cages measuring 6×6×6 inches and fed fresh diet cylinders twice daily. They were kept in an incubator at 85° F. and provided with 14 hours of light per day.

Oviposition data for a laboratory strain of aseptically reared boll weevils originally from Mexico and boll-reared adults from Mexico obtained in the field are given in table 2. Although there was considerable variation between groups of weevils, oviposition was much greater than that reported by Vanderzant et al. (1959) and in table 1. For 62 females fed the germinated cotton-seed diet a range of 61 to 198 eggs per female in a 20-day period was obtained, or an average of 103. Eighteen field-collected females laid an average of 152 eggs in 20 days. It is noteworthy that the laboratory colony had been reared for 25 generations or more when these tests were run. In spite of the long separation of the laboratory weevils from the field and the differences in larval diets, artificial for the laboratory weevils and bolls for the wild strain, the oviposition response was quite similar. Thirty-two females fed this diet with twice as much ascorbic acid laid an average of 149 eggs in 20 days. These results are lower than those obtained with squares in which 15 females laid 231 eggs per female in a 20-day period.

In two tests oviposition on a diet containing whole-square powder was similar to that obtained on a diet with cottonseed. The work involved in its preparation made this diet more costly than the diet with germinated seed.

Table 2.—Oviposition by aseptically reared boll weevils from Mexico on germinated seed and square-powder diets, and on squares.

Number of Females	Total Eggs per Female in			Number of Dead Females[a]
	20 Days	25 Days	30 Days	
Germinated Seed				
4	129			0
9	61			0
8	91			0
8	198	229		0
14	84	104	118	1 (26)
7	103			2 (10, 18)
12	91			0
16[b]	158	192	216	0
16[b]	139			2 (15)
8[c]	156	222	287	0
10[c]	148	183	210	1 (29)
Whole Square Powder				
5	95	112		2 (12, 22)
4	68			0
Acetone-Extracted Square Powder				
5	40			1 (16)
7	26			4 (10, 16, 17)
Squares				
15	231	307	383	0
9[c]	179	228		0

[a] Numbers in parentheses denote days on which females died.
[b] 0.4 g. of ascorbic acid added to 100 g. of diet.
[c] Wild strain, boll-reared.

A diet containing acetone-extracted squares gave poor results.

Adults reared from a leaf-meal diet containing antimicrobial agents (Brazzel et al. 1959) also were tested (table 3). These weevils were kept in an incubator with continuous light and provided with fresh food twice daily. A range of 72 to 218 eggs per female in a 20-day period was obtained with an average of 125 eggs per female, or 6.3 eggs per female per day.

Oviposition on a germinated cottonseed diet to which corn oil and fat-soluble vitamins were added was superior to all other artificial diets that have been tested (table 4). In 20 days, 96 females laid on average of 202 eggs each. Of eight groups tested only one laid fewer eggs than two groups of similar weevils on a diet without fat and fat-soluble vitamins (table 2). It cannot be concluded that the addition of these substances improved oviposition because there was a wide variation between groups of weevils. However, the inclusion of fat, fat-soluble vitamins, and additional ascorbic acid does not complicate the preparation of the diet or increase its cost appreciably and these substances apparently do not harm boll weevils.

Hatch of eggs from artificial diets was 72% or more, whereas hatch of eggs laid in squares was 85%. No changes in per cent hatch occurred during six consecutive generations of weevils fed purified diets as larvae and germinated-seed diets as adults.

Discussion.—Oviposition on germinated-seed diets was effective enough to make these diets practical for rearing. In spite of the care taken in conducting the tests, they were spread over a 12-month period and a number of factors contributed to the variations between different groups. Deltapine-15 variety of cottonseed was used in all tests, but different batches of germinated seed were used. Time, temperature, light, and humidity affect germination and these conditions were not always the same. Individual females differ in their egg-laying capacities. During the course of these tests, larvae of the laboratory strain of aseptically reared weevils were killed in an incubator with a faulty heat control and a new colony was started from the same parental line.

Table 3.—Oviposition on a germinated seed diet by adult boll weevils reared from leaf-meal diets.

Cage No.[a]	Total Eggs per Female in[b]		Number of Females Dead in 20 Days
	20 Days	30 Days	
1	149	229	1
2	218	317	1
3	72	—	3
4	123	—	1
5	133	—	0
6	92	—	0
7	106	—	1
8	105	—	0
Average	125	273	

[a] Five females per cage.
[b] Not corrected for mortality.

There are still several factors that must be worked out for maximum efficiency in preparation of the diet. Removal of the seed coats is a hand process. No satisfactory method of mechanical removal has been found. Germinated seed was compressed in a hydraulic press but the meats could not be separated from the seed coats. A laboratory-type cottonseed dehuller also failed to make the separation because of the high moisture content of the meats and the toughness of the seed coats. If the germinated seed was blanched in boiling water for 5 minutes, it was easier to remove the hulls by hand, but blanching did not improve mechanical separation. Furthermore, the manual method permitted the separation of the germinated from the ungerminated cottonseed. It is believed that a large per cent of ungerminated material will reduce the number of eggs laid because of the inferior nutritional quality of such seed.

A uniform preparation of germinated seed could be

Table 4.—Oviposition by aseptically reared boll weevils from Mexico on a germinated cottonseed diet containing corn oil and fat-soluble vitamins.

Number of Females	Total Eggs per Female in			Number of Lost Females
	20 Days	25 Days	30 Days	
8	170	218	258	0
8	233	290	326	2 (18)[a] (15)[b]
8	138	178	211	0
8	167	214	248	0
16	193	241	275	1 (14)[b]
16	202	248	282	1 (13)[b]
16	248	306	350	0
16	214	284	335	0
Average	202	255	294	

[a] Day on which one female died.
[b] Day on which one female escaped.

made by lyophilizing the material, grinding it into a fine powder, and using the powder in the adult diets. Under refrigeration the powder should remain stable for a long time. The dried material should be just as effective in promoting oviposition as the fresh homogenate.

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The Nonrandom Distribution of Foraging Honey Bees Between Apiaries[1]

WILLIAM R. LEE,[2] *University of New Hampshire, Durham*

ABSTRACT

How distance affects the distribution of foraging honey bees (*Apis mellifera* L.) was studied without having the results confounded by variation in the food supply. In the first experiment colonies were positioned at two locations 1,925 feet apart in a low-bush blueberry (*Vaccinium lamarckii* Camp) field; in the second experiment the colonies were 800 feet apart in an apple orchard. Bees from each apiary were distinguished by either genetic markers or radioactive phosphorus. The ratio of bees from one apiary to bees from the other was determined at various distances between the two apiaries. In both experiments the ratio of bees from Apiary A to bees from Apiary B increased significantly as the distance to Apiary A decreased. Regression analysis showed that this was due to both an increase in bees from Apiary A and a decrease in bees from Apiary B. Therefore, bees are not distributed at random in the area between apiaries, but, relative to the food supply, forage in greater numbers close to their hive.

Among pollinating insects the honey bee (*Apis mellifera* L.) is of unique value to modern agriculture because man can economically move millions of honey bees into an area of his choice. Since man selects the location of honey bee colonies, it is important to know the effect of distance on the distribution of foraging honey bees.

Boch (1956), by working with individually marked bees on artificial food sources, has shown that bees communicate availableness and quality of food sources in relation to distance; and, when food sources are equal, more foragers are recruited to a near source than to a distant one. However, difficulty has been encountered by other workers in determining the distribution of foragers from colonies containing 25 to 50 thousand individuals on natural food sources.

Levin et al. (1960) counted at various distances on alfalfa the number of honey bees per square yard, percentages of flowers tripped, flowers forming pods, seed yields, pollen-collecting bees, and rate of tripping by nectar collectors. They also measured such ecological factors as soil surface temperature, relative humidity, light reflected from a gray card on the ground, and sugar concentration in nectar taken from the honey stomachs of bees. They concluded that the effect of distance from colonies on the distribution of foraging honey bees could not be generalized because of the variation in ecological factors, although other workers reviewed by Levin et al. (1960) have made generalizations based on this type of data.

Unfortunately, any of the variables affecting plants may in turn affect the number of bees per unit area, per cent of fruit set, or yield. Factors affecting plant growth often occur in gradients across a field. While there are many statistical designs for determining the error caused by these gradients, it is not possible to use these designs in determining the distribution of foraging honey bees over a field, for the effect of distance from the apiary may be confounded with a gradient of some factor influencing plant growth.

This difficulty of uncontrolled variation may be overcome by using the percentage of marked bees from an experimental apiary to unmarked bees, if we assume that a given area is equally attractive to both marked and unmarked bees once they have arrived in that part of the field. Using this method on an apiary containing cordovan bees, Peer (1955) found that the percentage of cordovan bees decreased with increasing distance in all directions within a distance of 2½ miles from the apiary. Levin (1959) observed a rapid decrease in the per cent of both young and experienced cordovan bees foraging in one direction until few cordovan bees were observed more than 350 yards from the apiary; however, in another direction the percentage of cordovan bees 1¼ miles from the apiary was comparable to the per cent observed adjacent to the apiary. Levin (unpublished manuscript) observed the percentage of bees marked with Phosphorus-32 was least in the direction of a large apiary, greater toward two medium sized apiaries, and greatest in the direction of a small apiary.

In a preliminary experiment in an orchard 127 feet west of an apiary containing a cordovan colony the author

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observed a significantly higher ratio of cordovan to wild type bees than in a second apple orchard 522 feet farther west which contained a wild type colony.

From these experiments it is difficult to make general conclusions concerning the effect of distance because we do not know the origin of the unmarked bees used in computing the percentages. However, this problem may be surmounted by using the ratio of differently marked bees from two different locations rather than the ratio of marked to unmarked bees.

METHODS.—The genetic mutant cordovan has been used as a marker in studying the behavior of honey bees by Taber (1954), Peer (1955), Peer & Farrar (1956), and Levin (1959, 1960). This recessive mutant turns the black pigment a dark brown or cordovan but fortunately does not affect the yellow bands; therefore, it is possible to breed lines homozygous for cordovan which can be distinguished from each other by the number of abdominal yellow bands. In one of these experiments the author used a line homozygous for cordovan with three or more bands of yellow on the abdomen, hereafter called the light cordovan line; and another line homozygous for cordovan but with no yellow bands on the abdomen, hereafter called the dark cordovan line. All the queens were artificially inseminated.

Peer (1955) maintained a yard of bees homozygous for the cordovan gene and observed the bees foraging in appreciable numbers as far as 2½ miles from the apiary. He observed nothing unusual in their behavior or honey producing ability. Levin (1960) established 20 colonies, 10 with black Caucasian bees (marked with radioactive Phosphorus-32) and 10 with cordovan bees, in an alfalfa field. He observed that both types of bees seemed to show similar gradations in population as the distance increased. These observations indicate that the cordovan phenotype does not greatly affect the bees' foraging activity, and subtle effects that could have escaped detection would not likely alter conclusions drawn from the experiments here.

Bees were also marked with radioisotope Phosphorus-32 by using methods similar to those used by Levin (1960), except the instrument for detecting the radiation, a Tracer Lab. TGC-1 G.M. tube shielded from background radiation and connected to a scaler, was sufficiently sensitive to make radio-autograms unnecessary. Wings and legs from radioactive bees were found to contain very little of the radioactive isotope, confirming observations of Nixon & Ribbands (1952) that the isotope was internal; consequently, physical contact is not likely to produce contamination. To further eliminate the possibility of physical contact producing radioactive contamination, bees from check colonies were placed in containers with radioactive bees. After a week of storage at below 0° C. no contamination was found; therefore, separate containers for each bee are not necessary.

Meteorological data—temperature, estimated per cent of sky covered by clouds, wind velocity, and wind direction—were recorded. Wind velocity was determined by timing the movements of soap bubbles over a known distance. The wind was usually calm with an occasional gust as high as 7 miles per hour except during the first day of collecting on blueberry when gusts were 14 miles per hour from the west. The temperature was above 21° C., except for 19° C. to 20° C. the first and last days of collecting on blueberry and 14° C. to 20° C. the first and second days of observations on apple. Bees should have been able to orient themselves by the sun (von Frisch 1961) throughout these experiments, for either clear sky was visible or the position of the sun could be determined through thin haze.

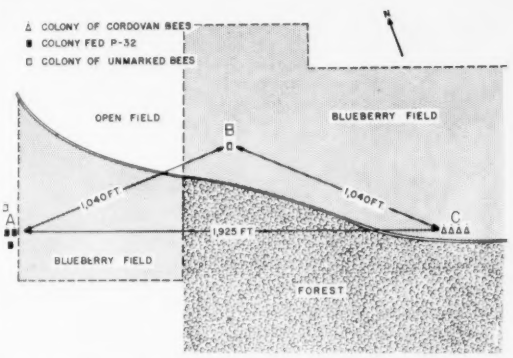

FIG. 1.—In the spring of 1958 colonies were located in low-bush blueberry fields as shown here.

1958 EXPERIMENT. Three colonies fed Phosphorus-32 were placed at location A in figure 1. A colony of unmarked bees was located at B, and four colonies containing bees homozygous for the cordovan gene were located at C. All colonies were moved into position a month before blossoms opened. Bees flying between A and C had to fly over a grove of trees; yet those flying to B from either A or C flew only over open fields of low-bush blueberries (*Vaccinium lamarckii* Camp). Bees were collected on blossoms of low-bush blueberry within 50 feet of colonies at locations A, B, and C on 4 different days. The collection periods were rotated among locations so as to randomize any effect variation in weather might have. The number of cordovan and radioactive bees collected at each location is shown in table 1. The ratio of radioactive bees to cordovan bees at location B is significantly different from both locations A and C ($P < 0.0001$). Therefore, the bees were not distributed at random with respect to distance over these two fields, but foraged in proportionally greater numbers close to their hives. A distance of only 1,000 feet is important to the honey bee under these competitive conditions. A very short flight range of slightly more than three-eighths mile between competing groups of colonies is indicated by this experiment. However, this was found

Table 1.—The numbers of cordovan and radioactive bees collected within 50 feet of the colonies located as in figure 1.[a]

LOCATION	FEET FROM LOCATION B	RADIOACTIVE BEES	CORDOVAN BEES	RADIOACTIVE BEES (%)	χ^2	P
A	1,040	114	1	99.13	74.88	<0.0001
B	—	46	50	47.92	—	—
C	1,040	3	119	2.46	63.68	<0.0001

[a] The chi-square test of homogeneity compares location B to both locations A and C.

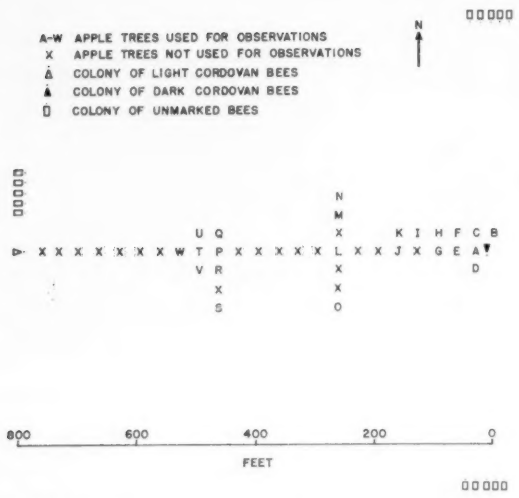

FIG. 2.—In the spring of 1959 colonies were located in a 40-acre apple orchard as shown here.

only in fields that had a great deal of competition from other colonies, for both radioactive and cordovan bees were found 2½ miles away in blueberry fields in which there were no colonies located.

1959 EXPERIMENT.—The following year an experiment was conducted to determine the effect of even shorter distances than those used in 1958 and also the effect of unequal competition in contrast to the nearly equal competition of the 1958 experiment. Accordingly, a weak overwintered colony containing 7,500 dark cordovan bees was placed in the center of a 40-acre apple orchard, and a stronger overwintered colony containing 17,000 light cordovan bees was placed 800 feet away at the edge of the orchard as shown in figure 2. (The number of bees per colony was determined by methods described by Farrar (1937).) The weak colony should furnish proportionally fewer bees than the strong colony (Farrar 1937). Fifteen, recently established, 3-pound package colonies were placed as shown in figure 2. All colonies were placed in the orchard before blossoms opened. Observations were commenced when the trees were in full bloom. For each observation I walked once around previously selected trees and observed the lower limbs only. Repeated observations on the same tree will improve the estimate of the ratio of bees attached to a particular tree, but will not continually improve the estimate of the ratio at that distance because of the limited number of bees attached to a particular tree. Therefore, no more than two observations were taken on any tree in 1 day, and no more than eight observations were taken on any tree during a 5-day period in May, 1959. Observations were taken only when bees were actively flying. Observations were spread throughout the sampling period; however, unavoidable interruptions caused an unequal number of observations on each tree.

Prior to conducting this experiment four trees (A through D) adjacent to the dark cordovan colony were selected as the standard. This group of trees was to be compared with groups L through O and P through W. The number of dark cordovan and the number of light cordovan bees in each of these three groups of trees are shown in table 2 along with the results of a chi-square test of homogeneity in which the standard is compared with the other two groups. The ratio of dark cordovan to light cordovan was very different from the standard at 292 and 522 feet, both chi-square tests showing a significant difference, ($P < 0.0001$). Therefore, the bees were not distributed at random over the distances used in this experiment.

Trees A through K provide a continuous array from 32 feet to 179 feet from the dark cordovan colony toward the light cordovan colony. The data given in table 3 show a sufficient number of both dark cordovan and light cordovan bees to permit analysis of the bees observed on each of the 11 trees.

Two Cortland trees were in the group A to K. To determine if these two trees biased the results because of varietal differences, the ratios of dark cordovan to light cordovan bees on Cortland trees E and J were compared, respectively, with the ratios on the adjacent McIntosh trees F and K. Neither the individual comparisons nor their total was significant. There was no interaction ($P = 0.3$), so the pooled data with a total of 87 bees can be used for a test of significance. The pooled chi-square value of 0.018 ($P = 0.90$) gives no indication of a significant difference in the ratio; therefore, the data from the two Cortland trees were pooled with the McIntosh trees.

A regression analysis based on the chi-square distribution as developed by Cochran (1954) was conducted by giving each tree a weight equal to its distance from the dark cordovan colony. The observed ratio of dark cordo-

Table 2. The ratio of dark cordovan to light cordovan bees in groups 2 and 3 is compared with group 1 with a chi-square test of homogeneity.[a]

Group	Trees	Average No. Feet from Dark Cordovan Colony	Cordovan Bees			χ^2	P
			Light	Dark	Per Cent Dark		
1	A through D	42.5	24	63	72.4		
2	L through O	292.2	88	12	12.0	70.68	<0.0001
3	P through W	521.5	105	0	0	113.13	<0.0001

[a] Group 1 was selected to be the standard before the experiment was conducted. (Tree symbols are the same as in figure 2.)

Table 3.—Number of dark cordovan and light cordovan bees observed on each McIntosh (McI.) and Cortland (Cort.) tree located as in figure 2.

Tree	Variety	Feet From Dark Cordovan Colony	Number of Cordovan Bees			Dark Cordovan Bees (%)
			Observations	Light	Dark	
A	McI.	32	6	9	15	62.5
B	McI.	42	7	8	18	69.2
C	McI.	43	5	6	12	66.7
D	McI.	53	7	1	18	94.7
E	Cort.	67	7	3	17	85.0
F	McI.	79	7	7	18	72.0
G	McI.	103	7	21	16	43.2
H	McI.	111	7	14	23	62.2
I	McI.	144	8	14	8	36.4
J	Cort.	175	5	13	10	43.5
K	McI.	179	6	10	9	47.4

van bees to the total number of marked bees observed on each tree is given a weight $n/\bar{p}(1-\bar{p})$, where n is the number of observed marked bees and $\bar{p}$ is the average ratio for the experiment. The ratio of dark cordovan to total marked bees differed among the 11 trees, for the chi-square value of 31.39 (d.f. = 10) was highly significant (P = 0.0005). The linear regression of the ratio of dark cordovan to total marked bees on distance had a chi-square value of 15.01 (d.f. = 1) which was highly significant (P = 0.0001) for even this short distance. The regression coefficient was −0.244 per 100 feet. Deviations from linear regression were close but not within significance (P = 0.06).

The ratio of dark cordovan to total marked bees is shown plotted in figure 3 for every tree in the experiment along with the regression line between the distances of 32 and 179 feet. With a relatively high concentration of bees at the light cordovan colony location the ratio was shifted in comparison with the 1958 experiment; instead of the 50% being half way between two apiaries, it is only 16% of the distance from the weaker one. A very short flight range of the dark cordovan bees of about 400 feet or less was observed in the direction of high competition in contrast to the 2,000 feet observed the previous year with equal competition over a greater distance. In the opposite direction from the light cordovan colony, dark cordovan bees were found as far away as 800 feet, the edge of the orchard. No observations were made outside this orchard.

A ratio was used rather than the number of bees per unit area so some uncontrollable, environmental variables would be factored out. It is interesting to compare the results obtained by this method of analysis with those obtained by computing the regression of number of bees on distance. Effort was made to observe a uniform area on

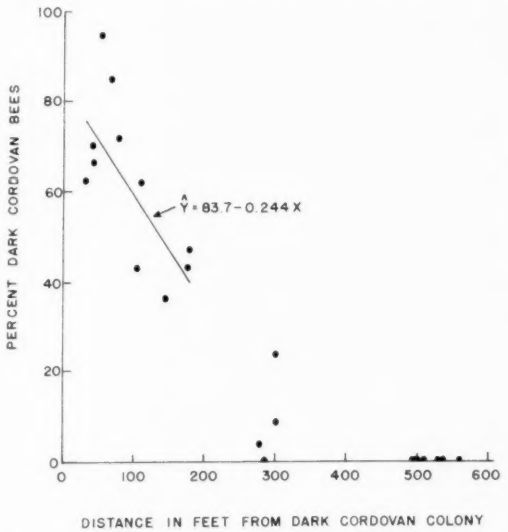

FIG. 3.—Dark cordovan bees (expressed as per cent of the number of marked bees per tree) are plotted against the distance of each tree (shown in figure 2) from the dark cordovan colony. The line is the weighted regression line between distances of 32 and 179 feet.

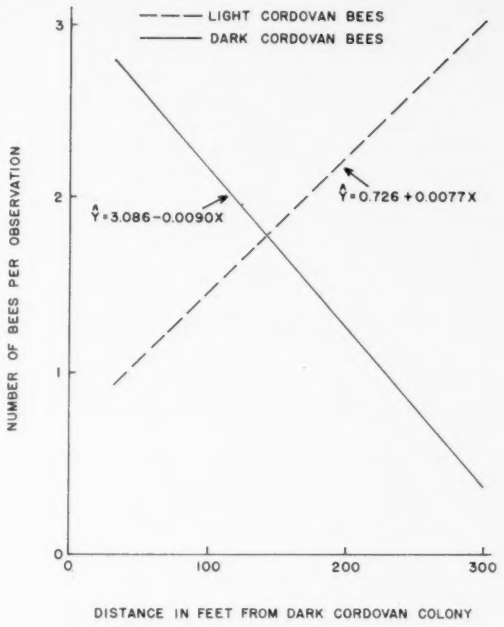

FIG. 4.—Weighted regression of number of bees per observation on distance was calculated for both dark cordovan and light cordovan bees.

each tree that was sufficiently close to the ground to permit accurate observations. A weighted regression analysis was run on the number of dark cordovan bees per observation on distance from the dark cordovan colony. The number of observations per tree was used as weights. The weighted regression coefficient is −0.73 dark cordovan bees per observation per 100 feet. This regression coefficient is close to significance (P = 0.056). A similar analysis on light cordovan bees gave a weighted regression coefficient of +0.95 light cordovan bees per observation per 100 feet, which was also close to significance (P = 0.057). If the analysis is run on trees A through O to include all the trees in which both dark cordovan and light cordovan bees were observed, the weighted regression coefficient of dark cordovan bees on distance from the dark cordovan colony is −0.90 bees per observation per 100 feet, which is highly significant (P < 0.0001). The weighted regression coefficient of light cordovan bees on distance from the dark cordovan colony for the same group of trees is +0.77 bees per observation per 100 feet, which is also highly significant (P = 0.0005). These two regression equations are plotted in figure 4. Since the regression coefficient of dark cordovan bees on distance from the dark cordovan colony is negative and significant while the regression coefficient of light cordovan bees on distance from the dark cordovan colony is positive and also significant, we can conclude that the shift in the ratio between the bees of these two colonies was due in part to both light cordovan and dark cordovan bees foraging in greater numbers close to their hive under these competitive conditions.

DISCUSSION.—It should be possible to predict the number of bees foraging in a given area from three param-

eters: the distance from the colony, the number of bees in the colony, and attractiveness of the area relative to attractiveness of other areas. While the first two parameters can be measured, the latter is particularly troublesome because we do not know all the variables that affect attractiveness. However, an increase in competition from either honey bees from other colonies or other nectar or pollen-gathering insects should decrease the attractiveness of an area. Thus, it is possible to decrease the relative attractiveness of an area even though we cannot now measure its value.

In order to study the way honey bees react to changes in distance without knowing the values of the other parameters, the change in ratio of foraging bees from two different apiaries was determined among sampling areas between the apiaries. Fortunately, it is not necessary to know the exact population in the competing colonies, as we are looking for a change in the ratio between sampling areas.

Weather may affect the ratio of bees from near to distant colonies, for observations by Butler et al. (1943) and Boch (1956) show that unfavorable weather will stop distant foragers while near foragers continue to work. The data in the 1958 and 1959 experiments were collected so as to randomize the effect of weather during the period the experiment was conducted; however, since more bees are collected under favorable than unfavorable weather conditions the data reported here should be interpreted as the reaction of honey bees to distance under favorable flight conditions.

The simplest hypothesis concerning the distribution of foraging honey bees is that they are distributed at random with respect to distance from their hive. From results of these experiments it must be concluded that at least one of the competing groups of bees did not distribute at random with respect to distance because of the significant change in the ratio. For, if a ratio between two variables changes, either one or the other variable must change, or both may change but not proportionally. The systematic change in the ratio of bees from competing colonies at different sampling areas between the colonies could be explained as a response to changes in competition which changed the relative attractiveness of the sampling areas, but there would be no change in competition between the colonies unless the bees from at least one of the colonies were foraging in larger numbers per unit area close to their hive.

The hypothesis that change in the ratio of competing groups of bees with changing distances is due entirely to behavior of one of the groups can be rejected; for in the blueberry experiment the number of cordovan bees was least in location A, intermediate at B, and greatest at location C; yet the opposite was true for the radioactive bees. In the apple experiment there was a highly significant decrease in the number of dark cordovan bees per observation over a distance of 268 feet between trees A and O; yet there was a highly significant increase in the number of light cordovan bees over the same distance. Therefore, the highly significant change in the ratio of dark cordovan to light cordovan bees between trees of group 1 and trees of group 2 must have resulted from a change in the distribution of both dark cordovan and light cordovan bees with different distances. It is likely that the highly significant, negative regression of the ratio of dark cordovan bees to the total number of marked bees on distance from trees A to K is also due to changes in the number of both dark cordovan and light cordovan bees; although the regression of dark cordovan and light cordovan bees per observation was not significant over this distance. This demonstrates that comparing ratios of bees from different locations is a more sensitive test for the effect of distance than comparing the change in number of bees per unit area.

Results of these two experiments show that bees from a colony decrease in number per unit area as the distance increases from their colony toward a competing colony, provided availableness and quality of food sources remain the same. Honey bees are very sensitive to small changes in distance to food sources; however, their sensitivity to changes in distances was greater in the apple experiment where there was apparently more competition. How changes in environment and heredity may affect the rate of decrease in number of bees per unit area with increasing distances is an open question. Whether or not the reaction of honey bees to distance is linear cannot be answered now because of the short distances over which these experiments were conducted and the fact that deviations from linearity were close to significance ($P=0.06$) in the chi-square regression analysis. If we assume that deviations from linearity were significant in the second experiment, it would not necessarily mean a nonlinear response to increases in distance; for other sources of variation like repeated counts of the same bee would contribute to the nonlinear source of variation.

The majority of a colony's foragers do not by themselves find new food sources; however, a small number of scout bees upon finding a new source of food recruit a number of foragers (Oettingen-Spielberg 1949, von Frisch 1961). If the probability of scouts locating the sampling area is low, the average response of foragers to distance will not be affected; but the variation among sampling areas will be increased. This could not have contributed to the variation among trees A through K in the apple experiment for bees from both colonies were working each tree.

Only those bees working flowers in the sampling area are recorded, as it is impossible to count bees fixed to this area which are in flight or in the colony. From Park's (1923) data, the time in flight for 300 feet should be about 15 seconds; therefore, the possibility that the change in ratio—with change in relative distance between competing colonies—is caused by an increased proportion of bees in flight that would not be counted, can be rejected for the 1959 experiment.

The ability of honey bees to adjust to small differences in distance can be explained by the work of Ribbands (1949) and Boch (1956). Boch found that the number of dances performed by a returning forager and consequently the number of bees recruited depend upon attractiveness and distance from the hive to artificial food sources. Ribbands (1949) observed considerable variation in attachment of honey bees to a particular foraging area. The frequent shifts in attachment were not randomized but directional. He considered the attached bees satisfied ones and the shifting bees dissatisfied ones changing in a direction of greater satisfaction. Therefore, there appears to be a feedback relationship between the value communi-

cated by returning foragers to new recruits and the satisfaction of the new recruits upon visiting the food source.

The short foraging range of bees in the area between competing colonies in contrast to bees from the same colonies observed foraging 2½ miles in a field with little competition can be explained. Initially the distant food source because of little competition has relatively high attractiveness; however, as other foragers are recruited to this distant source, the food supply available for each bee decreases and thus the area becomes less attractive. Recruitment decreases until an equilibrium is reached between new recruits and foragers being attracted elsewhere or dying. The level of foraging activity at equilibrium will vary with factors affecting plant growth, but foraging activity will be higher if the food source is close to the hive because distance is weighed against attractiveness in determining the value communicated by returning foragers (Boch 1956).

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Cyclical Changes in Numbers of Moths and Larvae of the Peach Twig Borer in California[1]

DOUGLAS W. PRICE and FRANCIS M. SUMMERS, *University of California, Davis*

ABSTRACT

Moth flight patterns of the peach twig borer (*Anarsia lineatella* Zeller), as well as the movements of larval broods into and out of foliage, fruits, and bark were studied during 1960 in two almond orchards in California. Three flights of moths were detected by means of a new sampling technique. The first flight, composed of moths resulting from the overwintered larvae, lasted from mid-April until early June. The second, composed of moths derived from first generation or May brood larvae, lasted from mid-June until late July. The third, which was much more pronounced than either of the two earlier flights, lasted from mid-August until October. The ability to sample moth populations presents possibilities for estimating levels of infestation and predicting impending crop loss.

It is known that during the spring and summer some larvae enter the bark and construct summer hibernacula. This study indicates that larvae which entered the bark during May, June, and July remained only temporarily. By the end of July most of these returned to the actively feeding stage. Larvae which entered bark during August and September remained through the winter. The data suggest that larvae which form temporary hibernacula early in the growing season are a possible source of infestation in stone-fruit orchards during the mid-summer harvest season.

The life history and habits of the peach twig borer, *Anarsia lineatella* Zeller, have been studied in California by a succession of entomologists since it was first recognized as an important peach pest in this State more than 70 years ago. The behavior of the pest is sufficiently well known for purposes of research on control methods (Bailey 1948, Summers 1955), but deficiencies still exist in knowledge of moth flight patterns and changes in numbers of bark- and twig-feeding larvae during successive generations. One of the weak points in control procedures is the lack of reliable methods for estimating population densities. The ability to measure levels of twig borer infestation and to predict impending crop damage are necessary adjuncts to assured control, especially when regional outbreaks occur.

It was possible during 1960 to chart for the first time the moth flights of the twig borer. Movements of larvae into and out of almond twigs, fruits, and bark also were studied. This information offers new direction to the search for practical methods of control, detection, and appraisal of twig borer populations.

MOTH FLIGHTS.—Attempts to trap twig borer moths in fermenting liquid baits or by means of various aromatic attractants have not been successful. During the course of

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experiments with traps, it was discovered that, when reasonably abundant, moths in repose on host trees could be counted directly. A procedure for sampling moth populations, based upon a direct count of moths observed per unit of time, was shown to be feasible. A majority of these dark-colored moths were found on the bole of the host tree, usually in greater numbers below the main crotch. Some were found on clods of dirt near the base of the tree. The number of moths counted in 30 minutes appeared to be a satisfactory sample of moth populations in unsprayed almond orchards. Trial runs, conducted in 1959, demonstrated that counts made in identical areas on successive days showed little fluctuation relative to overall seasonal variations. The moths on 15 to 20 trees were counted in each 30-minute interval.

This moth-sampling procedure was followed in two almond orchards throughout 1960. In one orchard, at Davis, mature trees were sampled by varieties; in another orchard, at Arbuckle, young trees were sampled without regard to variety. The same trees were sampled each time, at about 3- to 7-day intervals. In order to avoid counting the same moths twice, it was necessary not to disturb them, and to progress from tree to tree at about 2-minute intervals. Since cold and wind cause the moths to rest in folds and crevices, the sampling was restricted, as far as possible, to calm, mild days.

Moth counts made on Peerless variety trees at Davis during 1960 indicated three flights of moths (fig. 1, solid line). The first flight began in April and continued until mid-June. The maximum count was 26 moths per 30 minutes on June 2. A distinct break between this and the following flight was observed in this series of samples. The second flight of moths appeared after mid-June and continued until the end of July. The second flight attained its peak, 50 moths per 30 minutes, during mid-July. The third flight appeared early in August and continued until the middle of October. The magnitude of the third flight was striking; a maximum of 335 moths per 30 minutes was observed on August 17. The points scattered along the solid line in figure 1 show that comparable counts made on Nonpareil (·) and Texas (+) varieties in the same orchard conform with the counts made on Peerless trees.

Another series of flight curves was obtained in young 4-year-old almond trees at Arbuckle, about 40 miles north of Davis (fig. 1, broken line). These counts represent the number of moths observed in 30 minutes in a cross-row composed of NePlus, Nonpareil, and Texas variety trees. In this locality the interval between first and second flights was shorter, and the second flight began earlier. The moth counts at Arbuckle did not reveal a discrete third flight, although the counts for August and September were somewhat higher than those for June and July. The rise in numbers of first instar larvae recovered from bark samples which began early in August (fig. 3, lower line), aided in identifying the period of a third flight at Arbuckle.

LARVAE IN TWIGS AND FRUITS.—An earlier study (Summers & Price 1959) showed that the overwintered brood of larvae feed in the buds and developing shoots during February, March, and early April. The next generation of larvae usually is first detected in the twigs of host trees during the middle of May, and is termed the May brood. The buildup and decline of the May brood of larvae in twigs of young almond trees at Arbuckle during 1960 is shown in figure 2 ("twigs").

The buildup and decline of larval populations in almond fruits is shown for three varieties sampled at Arbuckle (fig. 2, "Nonpareil," "NePlus," and "Texas"). These varieties became susceptible to attack on successive dates during the summer in the order indicated. The downward slope of the curves for Nonpareil and NePlus varieties indicates the loss of their attractiveness to ovipositing moths as these fruits dried on the trees. Fruits of the Texas variety remained attractive until they were harvested in mid-October.

The occurrence of large numbers of larvae in almond fruits at a time when the twigs were essentially free of larvae suggests that the number of larvae in twig samples does not always give a reliable index of the twig borer population. The degree of attractiveness of the twigs, especially almond twigs, to the twig borer varies considerably; but in general it seems to decrease as shoot growth slows down during the summer and the terminals begin to harden.

LARVAE IN BARK.—It is well known that the peach twig borer overwinters as a partly grown larva within a hibernaculum which it constructs in the bark of its host tree. During the early spring, all of these immature larvae forsake their hibernacula and begin to feed on developing buds and shoots. The bark is then free of larvae until mid-May, when some of the newly hatched larvae of the May brood again burrow into the bark and construct summer hibernacula. It is known that some larvae of later summer broods also construct hibernacula. The length of time spent in an inactive state within summer hibernacula has not been studied extensively.

New data on cyclical fluctuations in the abundance of bark-infesting larvae were obtained by periodically sampling the bark of young almond trees at Arbuckle. Each bark sample consisted of a collection of bark chips, each of which contained evidence of one or more hibernacula. The presence of hibernacula was noted by small piles of frass deposited by the larva on the surface of the bark (Bailey 1948, Summers 1955). The sample size was determined by the number of such chips which could be cut during a 30-minute period.

Since larvae were found to construct as well as to vacate hibernacula throughout the growing season, the total number of hibernacula in the bark, including both occupied and vacant, appeared to increase as the season advanced. This increased abundance resulted in somewhat greater numbers of bark chips being cut as the season progressed. However, since it was not possible to know whether a hibernaculum contained a living larva until it was opened in the laboratory, the increased numbers of bark chips cut later in the season helped to offset the accumulation of vacated hibernacula in the bark sample. The number of bark chips cut during late May, June, and July ranged between 100 and 140; whereas, during August, September, and October, the number ranged between 140 and 200. Bark chips were cut from 15 to 20 trees during each sampling period.

The bark chips of each sample were dissected. The number of live larvae recovered on successive dates appeared to provide a rough estimate of changes in the bark-infesting larval population in the orchard. The upper line

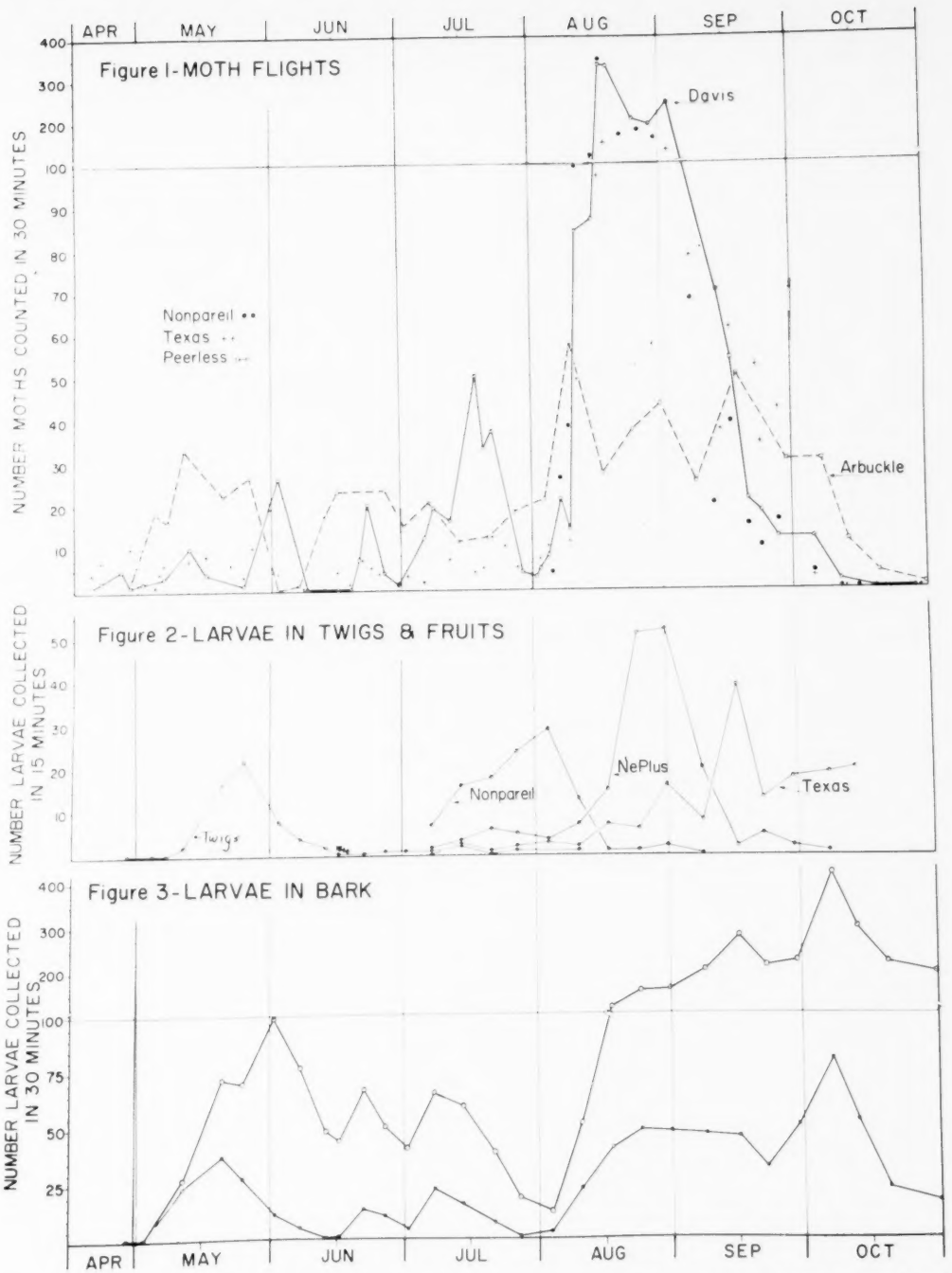

FIG. 1.—Numbers of peach twig borer moths counted per 30-minute sampling period on successive dates (1960) in almond orchards at Davis (solid line) and at Arbuckle (broken line), California. Counts of moths on Texas (+) and Nonpareil (·) variety trees at Davis are also shown.

FIG. 2.—Numbers of peach twig borer larvae collected per 15-minute sampling period on successive dates (1960) from almond twigs and fruits at Arbuckle, California.

FIG. 3.—Numbers of peach twig borer larvae recovered from samples of bark cut during 30-minute sampling periods on successive dates (1960) in an almond orchard at Arbuckle, California. The upper line represents total numbers of larvae per sample. The lower line shows numbers of first instar larvae in each sample.

in figure 3 shows the total number of live larvae present in each bark sample. The lower line of this graph shows the number of first-instar larvae in each of these samples, and thus reveals the periods of entry of hatching larvae into the bark. The number of dead larvae per sample during the period from May 11 until August 3 ranged from zero to 16, with an average of 8. From August 10 until October 31, the number of dead larvae ranged from 10 to 32, with an average of 17.

There were no larvae in the bark sample taken on April 28 and May 4; the only hibernacula cut were found to be empty remnants of the chambers of the overwintered larvae. On May 11, larvae of the first generation (the May brood) were found in the bark sample (fig. 3), as well as in twigs (fig. 2). This bark sample yielded 27 larvae, 23 of which were first instars. The remainder were second instar forms. The greatest number of larvae of the May brood were found in the sample cut on June 1 (fig. 3).

Larvae of the second generation appeared before mid-July, as shown by the samples of bark (fig. 3) and Nonpareil nuts (fig. 2). Despite the entry of new larvae of this brood into the bark during late June and July, there was an overall decline in the totals for bark-infesting larvae during these months. The irregular but downward trend in the numbers of larvae recovered from bark samples (fig. 3, upper line) indicates that most of the larvae which entered the bark phase during May, June, and July vacated their summer hibernacula sometime during the latter 2 months. The number of occupied hibernacula was found to be minimal near the end of July.

During the third flight period, August and September, there was again a buildup of bark-inhabiting larvae (fig. 3). These larvae, progeny of second- and possibly third-generation moths, remained in the bark and became the over-wintering generation. The bark samples showed a peak in total worm population during October. Thereafter the bark population declined gradually as shown by samples taken during the winter months (not included in graphs). The number of first instar larvae declined to zero in November.

DISCUSSION.—Eggs laid on foliage by moths of the first flight give rise to the so-called May brood of larvae. The dates on which the larvae of this brood are first detected in the twigs of peach and almond trees in the central valleys of California have been recorded for many years, since twig borer control programs depend upon the application of insecticides soon after these larvae are first detected. The average date of appearance of this brood is May 12 (Summers 1955).

The appearance of these first generation larvae in the foliage consistently during the middle of May leads one to expect that a flight of moths developing from this brood should also appear at about the same date each year. Since approximately 10 days are required for completion of metamorphosis during this part of the year (Bailey 1948, King & Denman 1960), moths should appear about 10 days after the first early-maturing larvae leave the foliage in May. The discrete nature of the second flight of moths observed at Davis during 1960 (fig. 1, solid line) suggests that this flight was composed largely of first-generation moths, that is, moths derived from May-brood larvae. The appearance of the second flight of moths at Arbuckle (fig. 1, broken line) was shown to follow the movement of the May brood of larvae out of the foliage (fig. 2, "twigs").

Since the twig borer life cycle can be completed in as little as 3 weeks during the warm summer months (Bailey 1948), it is probable that the strong third flight of moths which developed abruptly during the second week of August at Davis (fig. 1, solid line) was composed of more than a single generation of moths.

It may be practicable through refinement of this technique for sampling moth populations to determine levels of infestation in fruit orchards and to predict impending crop loss. Also, if it is found that discrete moth flights appear at regular intervals during the spring and summer months, it may be feasible to direct control operations to this phase of the life cycle.

The significance of larvae in summer hibernacula to crop damage has not been emphasized in prior studies of twig borer habits. Jones (1935) appreciated that larvae in summer hibernacula did not remain inactive, but that "after undetermined lengths of time these cells were seen to be abandoned." Jones (1935) also found that "the smallest number of summer hibernacula were found when ripe fruit was available." Bailey (1948) also appreciated that mid-season broods are "made up of two types of larvae; one that feeds on the bark and forms temporary hibernacula, then later migrates to fruit and completes its growth; and another that feeds entirely on green shoots and fruits."

The samplings done in this study suggest that a return of bark-infesting larvae to the actively feeding condition during a critical part of the fruit-growing season could be an unsuspected factor in crop loss. Severe crop damage during mid-summer has been observed in orchards, especially peach orchards, not showing appreciable twig damage or evidence of high-level moth activity earlier in the summer. The data obtained do not reveal how to equate numbers of bark-infesting larvae to the total population of larvae in an orchard. Nevertheless the possibility that bark-infesting larvae can augment the active larval population during the period of fruit ripening introduces the notion that insecticides which disinfect the bark as well as the foliage may be advantageous for use during May.

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Tests with Systemic Insecticides on Rice[1]

C. C. Bowling, *Texas Agricultural Experiment Station, Substation 4, Beaumont*

Abstract

Greenhouse and small plot tests were conducted in 1958 through 1960 with systemic insecticides to control a leafhopper, *Draeculacephala portola* Ball, on rice. In two greenouse tests, seed treatment with Di-syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphoroditioate), and phorate at 1 pound active ingredient per 100 pounds of seed, leafhopper control was obtained for approximately 30 days. The most effective control with granulated Di-syston in a greenhouse test was obtained when applied to the soil, flushed with water and flooded 24 hours later. Phorate was slightly more effective than Di-syston when applied to the soil as granules at the 2-pound rate. At the 4-pound rate, the two were equally effective.

In small plot tests, phorate and Di-syston when applied as granules broadcast at 2 pounds of active ingredient per acre, reduced leafhopper populations and increased yields. Phorate and Di-syston at 2 and 4 pounds active per acre applied as granules in the furrow at planting time produced increases in yield that were significant.

Since the discovery of the systemic action of certain organic phosphate insecticides, workers have reported on their effectiveness against insects on various field crops. Ivey et al. (1954) reported three systemics used as seed treatment to be effective against a wide range of insects on cotton. Reynolds et al. (1957) showed the effectiveness of certain systemics used as seed treatment and applications of granules in reducing populations of spotted alfalfa aphid, *Therioaphis maculata* (Buckton), on seedling alfalfa. Kantack (1955) reported on the effectiveness of several compounds applied as seed treatment and as a soil trench method on wheat to control the wheat curl mite, *Aceria tulipae* (Keifer). Bowling (1957) found that phorate used as a seed treatment was ineffective in controlling the rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel), on rice.

The spread of the planthopper, *Sogata orizicola* Muir, in Cuba, several South American countries and Florida, as reported by Atkins & McGuire (1958) prompted preliminary studies on the use of systemic insecticides on rice in 1958. Atkins et al. (1960) reported the presence of this insect and the hoja blanca disease which it transmits in the major rice-growing area of Louisiana. This problem indicated a need for further work with systemic insecticides on rice.

Greenhouse Tests with Seed Treatment.—One-pound lots of Bluebonnet 50 seed rice were treated in June 1958, with insecticides at the rate of 1 pound active ingredient per 100 pounds of seed. The insecticides used were as follows: Di-syston® 50% (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate); phorate 44%; Tetram® 50% (*O,O*-diethyl *S*-(β-diethylamino)-ethyl phosphorothiolate (hydrogen oxalate salt); Shell SD-3562 (2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate).

Each insecticide was impregnated on charcoal dust as suggested by Ivey (1952). Methocel sticker was used at the rate of 6 ounces per 1,000 pounds of seed. Treatment was made by placing the seed and sticker solution in a 1-gallon jar and rotating until coverage was obtained. The insecticides were then added and the jar rotated again until the powder adhered to the seed.

Ten seed from each treatment and check were planted 1″ deep in 6″ pots containing Beaumont clay soil (one of the major soil types in the Texas rice-growing area). The plants were watered from the bottom by placing in shallow soil flats containing 2″ water. The test was replicated four times. One week after planting, the plants in each pot were thinned to four per pot. Each pot containing the plants was then enclosed separately with plexiglass cylinders as shown in figure 1.

Fig. 1.—Rice plants enclosed with plexiglass to confine leafhoppers to various systemic insecticide treatments.

A leafhopper, *Draeculacephala portola* Ball, commonly found on rice in Texas, was used as the test insect in all experiments. Adults were collected by sweeping from rice or from grass, depending on abundance and ease of collecting. The leafhoppers were transferred from the net to a gallon jar and anesthetized with ether for counting. They were then placed in 90-mm. shell glass vials, plugged with cotton and allowed to recover. Each pot was infested with 10 adult leafhoppers. Survival counts were made by visual observation 48 hours after exposure. Following these records, the covers were removed and the survivors allowed to escape before the next infestation was made. Data from each test are shown on bar graphs. Analysis of variance was calculated on data at each infestation date.

Data from Test No. 1 shown in figure 2 indicate that Tetram was not taken up in sufficient quantities to affect the survival of the test insect. Phorate and Di-

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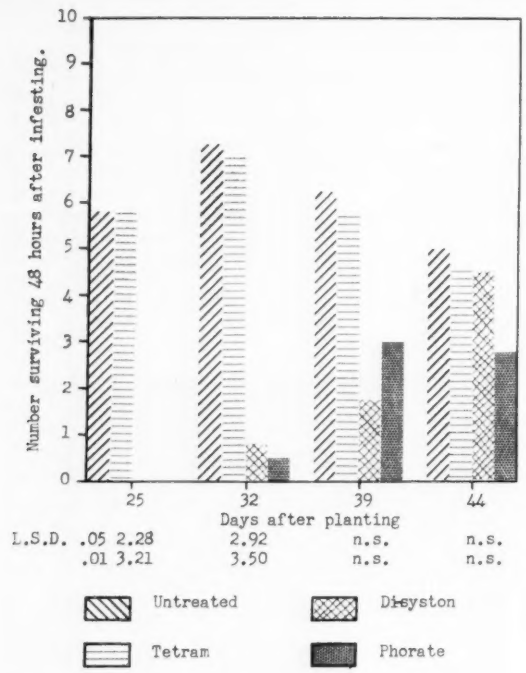

FIG. 2.—Survival of leafhoppers on rice grown from seed treated with systemic insecticides in Test No. 1.

syston were equally effective in reducing survival for 32 days from date of planting.

Test No. 2 was conducted in the same manner as Test No. 1, but the seed were planted at a later date. Data are shown in figure 3. Shell SD-3562 was only slightly effective in reducing the number of insects, while phorate and Di-syston were equally effective for 43 days.

GREENHOUSE TESTS WITH GRANULATED MATERIALS.—Two greenhouse tests with applications of granules were conducted in 1959. The first test was conducted to determine the effect of soil moisture condition on the uptake and retention of Di-syston. Ten Bluebonnet 50 seed were planted per 6″ pot in the greenhouse. Two weeks following date of planting, each pot was thinned to 4 plants per pot and 3 weeks later an application of Di-syston 5% granules was made at the rate of 2 pounds active insecticide per acre. The treatment variable was the moisture condition of the soil at time of application. The various moisture conditions were obtained by first placing the pots of enclosed rice plants in 3-gallon crocks. The treatments were applied as follows:

(A) With soil flooded with 2″ water.
(B) To saturated soil and allowed to dry 24 hours before flooding.
(C) To dry soil and flooded immediately with 2″ water.
(D) To dry soil, flushed and then flooded 24 hours later.
(E) Untreated check.

After initial moisture condition was obtained, all pots were kept flooded with 2 inches of water throughout the test period.

Data from this test shown in figure 4 indicate that treatment D (application made to dry soil, flushed and then flooded 24 hours later) was the most effective in controlling the leafhoppers.

The second test was designed to determine the effectiveness of Di-syston and phorate at 2 and 4 pounds of technical material per acre. The same experimental procedure was followed as in the previous tests with granules. All applications were made to dry soil, flushed and allowed to dry 24 hours and then flooded. Data shown in figure 5 indicate that all treatments reduced leafhopper populations below the untreated check for 29 days. All treatments except Di-syston at the 2-pound rate reduced populations below the check for 36 days. Up to 22 days there was no difference between the effectiveness of the two insecticides or the two rates applied. Phorate at the 2-pound rate was more effective than Di-syston at the 2 pound-rate at 29 and at 36 days.

SMALL PLOT TESTS WITH GRANULATED INSECTICIDES.—A small plot test was conducted in 1959, using plots 4′×20′ randomized with four replications. Phorate and Di-syston 5% granules were used at the rate of 1, 2 and 4 pounds technical insecticide per acre. Applications were made 3 weeks after planting. A fertilizer application of 100 pounds nitrogen per acre was made immediately after the insecticide treatments and the plots were then flooded with 2″ of water.

Counts of the rice water weevil were made 3 weeks after application date and no significant difference between insecticide treatments and checks was found. Leafhopper

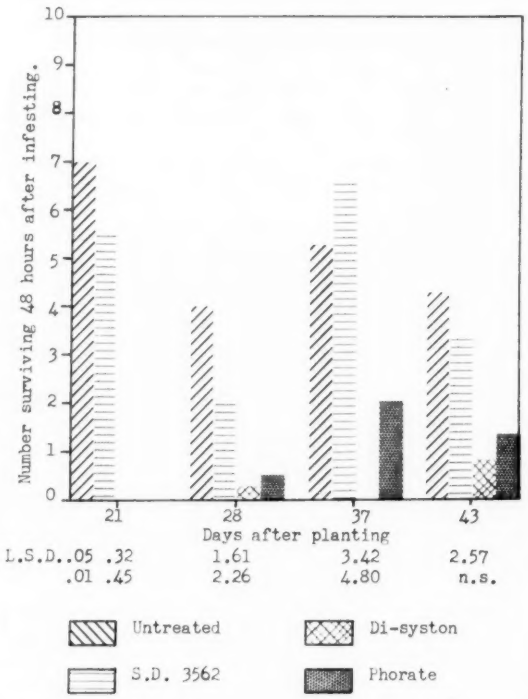

FIG. 3.—Survival of leafhoppers on rice grown from seed treated with systemic insecticides in Test No. 2.

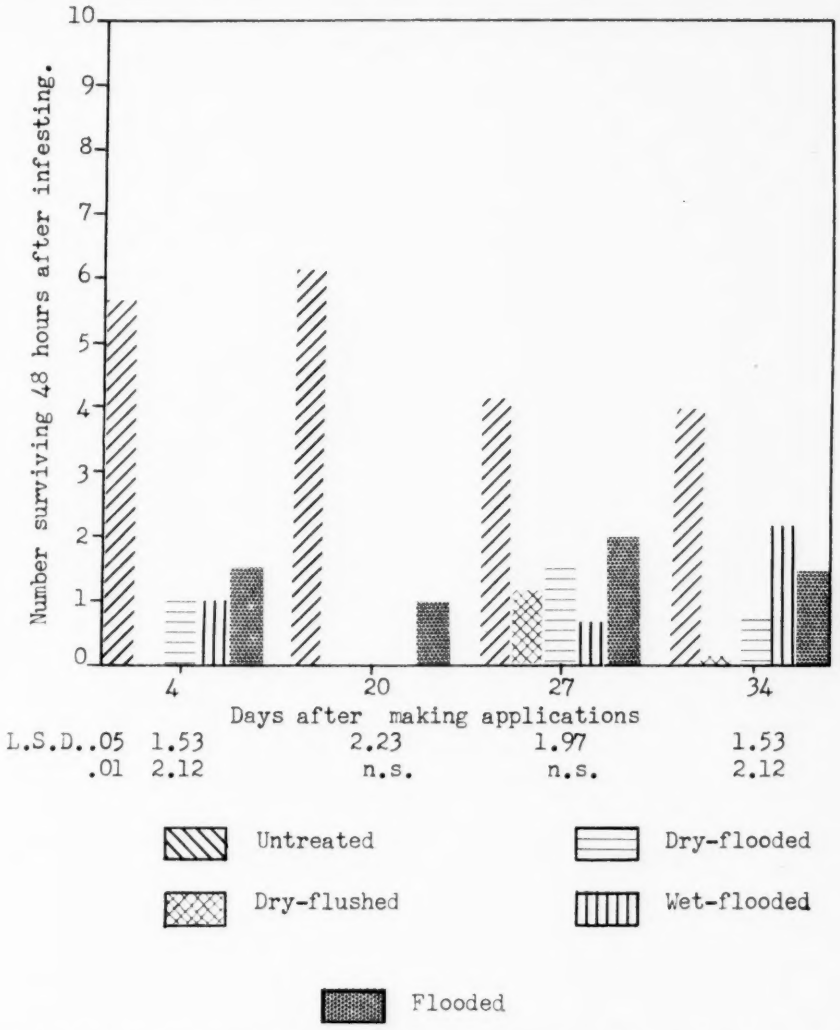

FIG. 4.—Survival of leafhoppers on rice treated with granulated 5% Di-syston at the rate of 2 pounds technical per acre. Treatment variable was moisture condition of soil at time of application and irrigation practices.

counts made by sweeping with an insect-collecting net indicated no difference between treated and untreated areas. It is believed that the flood caused a lateral dispersion of the insecticides into the untreated checks. Dead adult leafhoppers were seen floating on the surface of the water on several occasions. Yield data indicated no differences among treatments and checks.

A small plot test was conducted to determine the effectiveness of phorate and Di-syston in controlling a natural population of *Draeculacephala portola* Ball in 1960. Both insecticides were applied as 5% granules broadcast at 2 pounds active ingredient per acre. Applications were made to dry soil with a Gandy distributor 3 weeks after date of planting. In this test, each 1/10-acre plot of Gulfrose was separated by levees to prevent lateral movement of the insecticides. The two treatments and check were randomized within two replications. The plots were flooded immediately after making the applications and remained flooded until drained for harvest. Leafhopper counts were made by sweeping with a 15" insect-collecting net. Forty sweeps were made while the sweeper was walking diagonally across each plot. Yields are reported as pounds of cleaned rough rice per acre. Results of this test reported in table 1 indicate that both insecticides reduced the leafhopper population. Phorate was more effective than Di-syston. Yields were increased with both insecticides, but the increases were not significant.

SMALL-PLOT TEST WITH FURROW APPLICATION OF GRANULES.—A small-plot test was conducted in 1960 using 5% phorate and 5% Di-syston granules applied in the drill row below the seed at planting time. Treatments were randomized within each of four replications. Plot

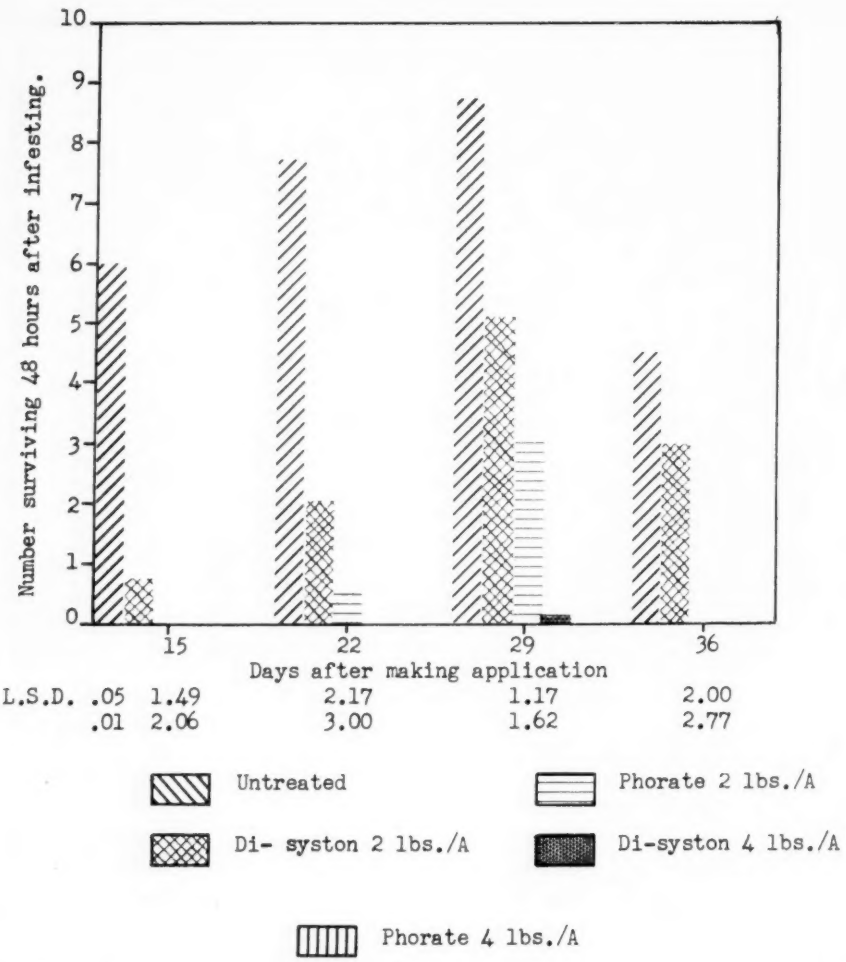

FIG. 5.—Survival of leafhoppers on rice treated with granular applications of systemic insecticides in Test No. 4.

size was 4′×20′. The variety of rice was Century Patna 231 seeded at the rate of 80 pounds per acre. The seed were treated with 75% aldrin wettable powder at the rate of 4 ounces active per 100 pounds of seed to control the rice water weevil. The seed were planted July 3 and an application of ammonium sulfate and superphosphate was made at the rate of 80–40–0 per acre, 3 weeks after planting. A flood was applied immediately after fertilization and maintained until drained for harvest.

Table 1.—Results of small-plot test to control leafhoppers on rice with systemic insecticides.

Insecticide[a]	No. Leafhoppers/40 Sweeps			Yield (lb./acre)
	July 22	July 29	Aug. 5	
Phorate	9	2	16	3,181
Di-syston	41	22	36	2,822
Check	68	61	78	2,632

[a] Each insecticide applied as 5% granules broadcast at the rate of 2 pounds active ingredient per acre.

The treatments had no visible effect on the germination and emergence of the plants. Several attempts were made to determine leafhopper populations on each plot by sweeping; however, no difference could be established because of the small size of the plots. Dead leafhoppers observed floating on the surface of the water were presumably killed by the insecticide treatment. Visible differences in plant height appeared among checks and all treatments when the rice reached the "boot" stage; however, these differences vanished after culm elongation. Yield data shown in table 2 indicate no significant differences in effectiveness between the insecticides. Yield increases were significant only between 0 and the 2-pound rate and 0 and the 4-pound rate. Since differences in leafhopper population were not established between treatments, it is possible that other unknown factors might be responsible for differences recorded in yield.

Results obtained from these tests indicate that phorate

Table 2.—Yields of rough rice from test to control leafhopper with systemic insecticides applied as granules in the furrow at planting.

Insecticide	Active Ingredient per Acre (lb.)				Average
	0	1	2	4	
Phorate	2,943	3,051	3,168	3,395	3,139
Di-syston	2,853	3,219	3,509	3,301	3,221
Average	2,898	3,135	3,339	3,348	

	Rates	Insecticides	Rates×Insecticides
L.S.D. at 5% level	307	N.S.	N.S.
at 1% level	[N.S.]	N.S.	N.S.

and Di-syston are absorbed and translocated in quantities that are toxic to the leafhopper, *Draeculacephala portola* Ball. It is believed they might be effective in controlling the planthopper, *Sogata orizicola* Muir, in the event this insect becomes a pest on rice in the United States.

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A Comparison of Co-Ral, Ronnel, and Ruelene Dusts for Screw-Worm Control[1]

M. J. Wrich, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

Abstract

Livestock shipped into the Southeast from areas infested with screw-worms (*Callitroga hominivorax* (Coquerel)) are examined and treated with insecticide at livestock inspection stations operated by the U. S. Department of Agriculture. Tests were undertaken to develop a dust treatment as an alternative for spraying so that wetting of the animals in cold weather could be avoided. In experiments using artificially infested animals, power dusting was as efficient as hand dusting, and Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) dust was more effective than Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate) or ronnel dusts in killing screw-worms and preventing reinfestation for 48 hours.

In 1958, the Animal Disease Eradication Division (ADE) of the U. S. Department of Agriculture and the Florida Livestock Board began an extensive campaign to eradicate the screw-worm (*Callitroga hominivorax* (Coquerel)) from Florida and the other southeastern States by the systematic release of sterile male flies (Bushland 1960). Federal regulations were issued to prevent the shipment of screw-worm-infested livestock into the eradication zone; included in this phase of the program was the establishment of 13 livestock-inspection stations located along the Mississippi and Pearl Rivers so as to intercept the principal east-west highways between Memphis, Tenn., and the Gulf of Mexico.

All livestock entering the eradication area through these inspection stations are unloaded and chute-inspected for screw-worms. All wounds are smeared with EQ-335 wound dressing. With the exception of a few classes of livestock, all animals are thoroughly sprayed with 0.25% Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) to kill young larvae that might not be noticed in routine inspection.

Insecticides used at these stations must be toxic to larvae up to 2 days old, since it is believed that older larvae can be detected by visual examination of the animals, and must have sufficient residual effect to kill larvae hatching from eggs present at the time of application (Wrich & Bushland 1960, Division of Insects Affecting Man and Animals 1951). The treatments employed are effective for this purpose, but because of possible ill effects to wet animals being moved in trucks during cold weather, ADE requested that the Kerrville, Texas, laboratory of the Entomology Research Division develop a dust treatment as an alternative in certain instances to the spray application used at the inspection stations.

Methods and Materials.—Co-Ral and ronnel were selected for exploratory tests because of the excellent results obtained with these materials when they were used as sprays in controlling screw-worms under laboratory and field conditions (Brundrett & Graham 1958, Graham et al. 1959). Results of the preliminary dust trials with cattle indicated that 5% Co-Ral applied with a rotary hand duster met inspection-station requirements. In subsequent tests the efficiency of an electric-powered duster was compared with that of the hand duster, and ronnel and Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate) dusts were compared with Co-Ral.

Yearling cattle of mixed breeds were wounded by removing a circular piece of skin about 1 inch in diameter and scarifying the exposed muscle tissue; the wounds were

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then implanted with about 200 newly hatched screw-worm larvae. Each animal was wounded and infested in an exposed and a hidden location on each of 4 successive days, so that at the time of treatment the larvae in the 8 wounds ranged in age from 4 hours to 3 days. The exposed wounds were made on the shoulder, back, brisket, and belly, and the hidden wounds in the front flank, inside the rear flank, within the ear, and 6 inches below the base of the tail. After the first test, a hip wound and another belly wound were made instead of the tail and ear wounds because it was difficult to establish larvae in those locations. After the tests with ronnel, larvae were not implanted on the day of the treatment because it had been determined that all the treatments were toxic to 4-hour-old larvae.

Sheep and goats were wounded in three exposed locations, and at the time of treatment one of two wounds on the hip contained 2-day-old larvae, the other 3-day-old larvae, and a shoulder wound contained 1-day-old larvae.

A chute was employed to confine the livestock during the treatments, and enough insecticide was applied to cover each animal entirely; the animals were examined daily after treatment. Most of the larvae were affected within 10 minutes and were killed or driven from the wounds within 4 hours. A treatment that failed to kill larvae up to 2 days old after 24 hours was usually discontinued, and the animal was removed from the test. An effective treatment was challenged by introducing about 200 newly hatched larvae on a new raw spot made by debriding a circular ½-inch area in each wound. The animal was examined on the following day, and if larvae had become established in the wounds the animal was removed from the test. If larvae survived in only one wound and not the others, these larvae were killed with benzene and that wound was not used further. (Benzene was used because it does not leave any residue.) Each wound was

Table 1.—Results of eight series of tests comparing Co-Ral, ronnel, and Ruelene dusts for screw-worm control.

Insecticide and Concentration (%)		Number of Cattle Treated	Number of Wounds Containing Larvae of Indicated Age at Time of Treatment				Number of Wounds With 100% Mortality of Larvae of Indicated Age at 24 Hrs. Posttreatment				Number of Wounds Reinfested at Indicated Days Posttreatment					
			4 Hrs.	1 Day	2 Days	3 Days	4 Hrs.	1 Day	2 Days	3 Days	1	2	3	4	5	6
						Series 1[a]										
Co-Ral	0.5	1	2	2	2	2	2	2	0	0	0	1	2	0	0	0
	1.0	1	2	2	2	2	2	2	2	0	0	1	3	0	0	0
	2.0	1	2	2	2	2	2	2	1	1	0	1	1	0	0	0
	5.0	1	2	2	2	2	2	2	2	2	0	0	0	0	0	1
						Series 2[a]										
Co-Ral	5.0	10	23	25	21	10	23	25	17	10	0	0	0	0	4	0
						Series 3[b]										
Co-Ral	5.0	20	40	40	40	40	40	40	37	38	0	0	1	0	0	6
						Series 4[b]										
Co-Ral	5.0	6	12	13	11	12	12	13	11	12	0	0	0	0	0	0
Ronnel	5.0	7	14	14	14	14	14	14	14	13	0	0	0	0	0	0
	10.0	7	14	14	14	14	14	14	12	14	0	0	0	0	0	0
						Series 5[b]										
Ronnel	5.0	13	26	26	26	26	26	26	26	26	0	1	0	0	0	0
						Series 6[c]										
Co-Ral	5.0	10	—	20	20	20	—	20	20	19	0	0	2	9	0	5
		5[d]	—	5	5	5	—	4	4	5	0	0	1	0	0	1
		5[e]	—	5	5	5	—	4	5	5	0	0	0	1	0	0
Ruelene	5.0	2	—	4	4	4	—	4	4	4	0	0	1	2	0	1
		2[d]	—	2	2	2	—	2	1	2	0	0	1	1	0	1
		1[e]	—	1	1	1	—	1	1	0	0	0	0	0	0	0
						Series 7[e]										
Ruelene	5.0	8	—	16	16	16	—	16	16	15	8	—	—	—	—	—
						Series 8[f]										
Co-Ral	5.0	10	—	20	20	20	—	20	20	20	0	0	8	3	0	0

[a] Hand duster, outside.
[b] Power duster, outside.
[c] Power duster, in unventilated building.
[d] Sheep.
[e] Goats.
[f] Power duster, enclosed chute.

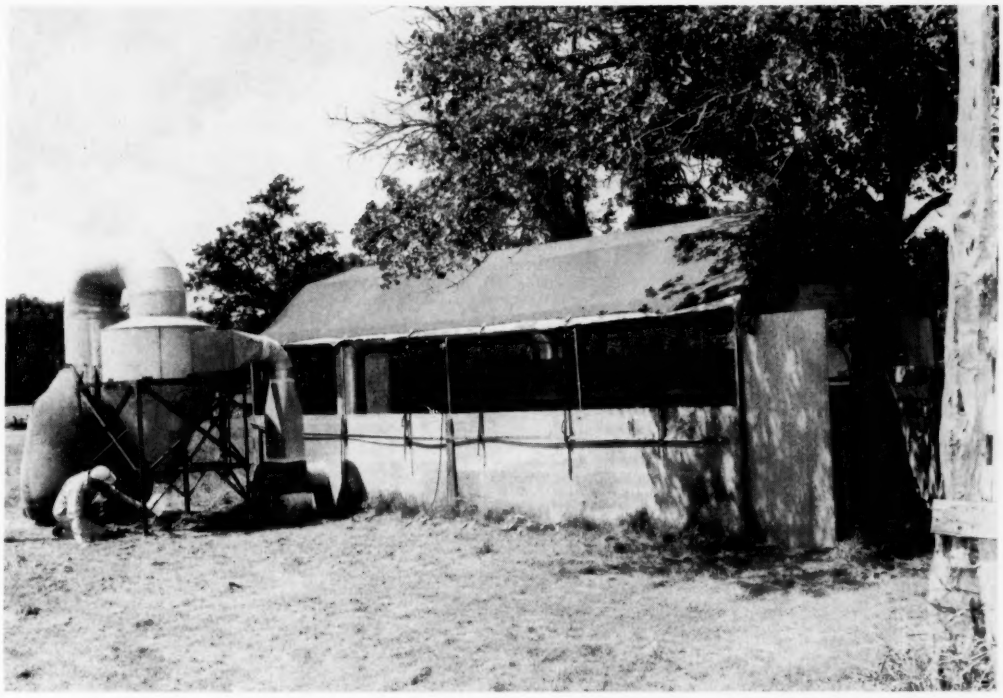

FIG. 1.—Outside view of dusting facilities and cyclone dust-collector.

challenged each day for 7 successive days, or until it became reinfested.

TESTING AND RESULTS.—In all, eight series of tests were conducted using various concentrations of the three insecticides, different equipment, and different arrangements for the dusting operation. The results are presented in table 1.

Series 1.—In this preliminary trial four yearling Herefords were treated to compare the effectiveness of 0.5%, 1%, 2%, and 5% Co-Ral dusts. The animals were confined in an outdoor chute and about 10 ounces of dust was applied to each with a rotary hand duster. Because of difficulty in maneuvering the delivery tube of the duster between the boards of the chute and because of moderate winds on the day of treatment, about half of the quantity applied was lost through drift. Only the 5% concentration was completely effective against larvae of all ages after 24 hours.

Series 2.—A second group of cattle of mixed breeds was similarly treated; this time only the 5% Co-Ral dust was used. All the larvae in 75 of 79 wounds were killed. Some 2-day-old larvae survived in hidden wounds because they had burrowed under the skin away from the opening of the wound and out of reach of the insecticide. However, newly hatched larvae failed to become established when the treatment was challenged after 48 hours.

Series 3.—This and subsequent tests were conducted with a modified electric-powered duster to compare its efficiency with that of the hand duster used in the first two series.

Twenty cattle were treated in the outdoor chute with about 2 ounces of 5% Co-Ral dust. The nozzle was held about 3 feet from the animal, and it was determined that an average of 1½ ounces of the dust adhered to the hair and penetrated to the skin. This treatment was toxic to all the larvae in 155 of 160 wounds. In the five remaining wounds, surviving 2- and 3-day-old larvae, estimated at about 15 per wound, formed pockets out of reach of the insecticide. The treatment was effective for 48 hours. The results of this test indicated that power dusting was as effective as hand dusting, and more economical, since satisfactory coverage was obtained with a smaller quantity of dust.

Series 4 and 5.—Two tests similar to Series 3 were conducted with three groups of cattle to evaluate ronnel dusts. In Series 4, seven steers were treated with a 5% concentration and seven with a 10% concentration. In Series 5, 13 additional cattle were treated with a 5% concentration and the results compared with those of the previous test. Both the 5% and 10% concentrations were toxic to 4-hour- and 1-day-old larvae, but neither was effective against all ages. Some larvae became established after 48 hours in one wound treated with the 5% dust. After 8 days live larvae were found in 9 of 160 wounds challenged. The 10% treatment was effective through 6 days, at which time 2 of 56 wounds became reinfested. On the other hand, all the larvae in 48 control wounds treated with 5% Co-Ral were killed and all wounds were protected for 6 days.

Series 6.—In another experiment, the effectiveness of 5% Ruelene was compared with 5% Co-Ral. Ruelene was applied to 2 cattle, 2 sheep, and 1 goat, and Co-Ral to 10

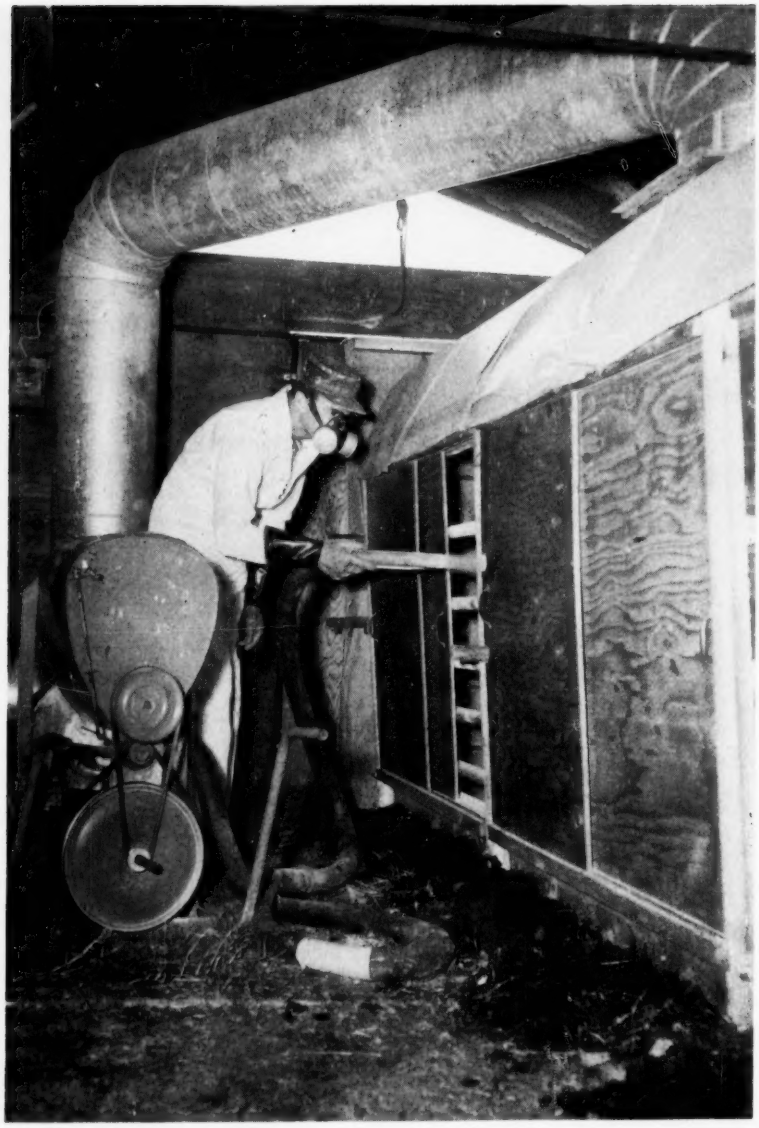

FIG. 2.—Application of dust to livestock confined in enclosed chute.

cattle, 5 sheep, and 5 goats. In contrast to the previous tests, the animals were confined in a chute inside a small building constructed specifically as an enclosure for the dusting operation. The building was not ventilated in order to avoid contamination of surrounding grounds and buildings with the insecticide. Because the air did not circulate, there was a heavy suspension of dust in the entire building which reduced visibility and hindered the operation; application of the material to cattle was uneventful, but the sheep had to be held and treated individually because the cloud of dust made the animals crowd together. Each of the six groups of animals treated was held inside the building for about 7 minutes to allow the dust to settle.

Co-Ral was toxic to all larvae in the cattle, except for a few in one hidden wound in the front flank, and the insecticide prevented reinfestation of all wounds for 48 hours. Ruelene also killed all larvae in all wounds on the cattle, but results were inconsistent on the other animals. Live larvae were found in the Ruelene-treated cattle and sheep after 48 hours.

Series 7.—The effectiveness of Ruelene against all ages of larvae in cattle in the previous test prompted additional work with eight more cattle. The initial toxic effect

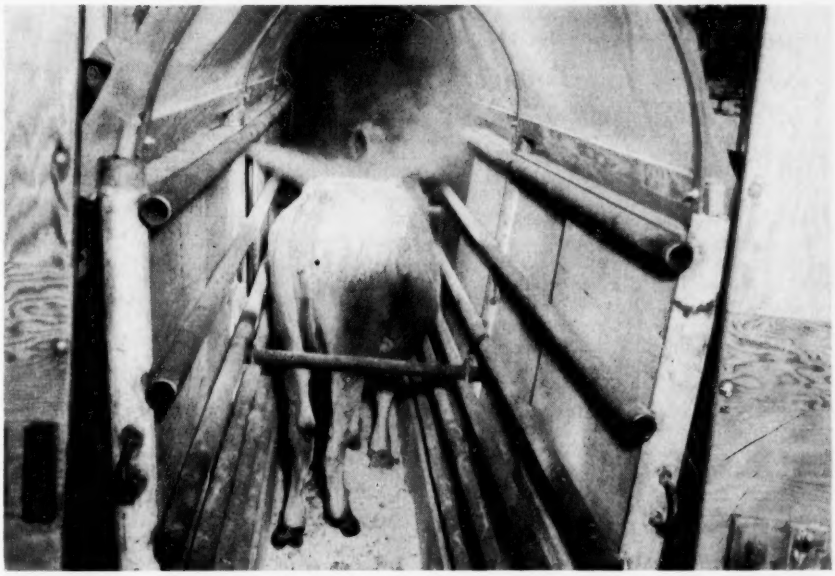

FIG. 3.—Inside view of enclosed chute.

of the treatment was almost equal to that in the previous test; in only one shoulder wound, which contained about 50 surviving 3-day-old larvae, did the treatment fail. All of the wounds were challenged at 24 hours posttreatment, and larvae became established in eight of these. This lack of residual effectiveness after 24 hours caused the test to be terminated.

Series 8.—For this test the chute in the dusting building was enclosed with sliding plywood panels on the sides and with polyethylene over the top to confine the dust. Burlap bags were used as curtains at each end. Air and suspended dust particles were removed from the enclosure with a squirrel-cage blower (2 hp.) moving 3,700 cubic feet of air per minute at 1 inch specific pressure, and passed through a cyclone dust-collector. A breather bag was installed on the cyclone exhaust to collect the remaining very fine dust particles that would otherwise have escaped and contaminated the area (see fig. 1).[2]

Before the cattle, sheep, and goats were driven into the chute, the sliding panels were opened to minimize the animals' resistance to entering. Once they were inside, steel poles were inserted behind each animal to separate it from the others, and all the panels were closed except one. The nozzle of the duster was directed through this opening to treat an individual animal with about 1¼ ounces of 5% Co-Ral dust (see figs. 2 and 3). The air current pulled the dust away from the operator at the open panel and prevented the dust from escaping into the building. This circulation of air seemed to increase the uniformity of dust coverage on individual animals. Before the operator moved to the next panel, the duster was turned off and the first panel was closed. In these tests the operation required about 13 minutes for each group of four animals.

DISCUSSION.—Corollary observations on the mammalian toxicity of the three insecticides used in the eight series of tests were made by personnel[3] of the Animal Disease and Parasite Research Division's laboratory at Kerrville, and the report of their research will be published elsewhere. It appeared that the safety of the dust applications compared very favorably with that of sprays formulated from the same insecticides.

In the tests conducted, the dust containing 5% Co-Ral was consistently superior to 5% Ruelene and 10% ronnel dusts in residual effectiveness. The test data clearly indicated that this 5% Co-Ral treatment applied with an electric-powered duster in an enclosed and ventilated chute was as effective as the standard spray treatment, and had the advantage of protecting the operator and personnel in the area from exposure to the insecticide. In 1959 the dust and similar dusting chutes were adopted for use at several of the screw-worm inspection stations.

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[2] This equipment was designed by C. N. Husman, Animal Disease Eradication Division, U. S. Department of Agriculture.

[3] R. D. Radeleff and L. M. Hunt.

Resistance in *Nicotiana* to the Green Peach Aphid and Some Other Tobacco Insect Pests[1]

RICHARD THURSTON, *Entomology and Botany Department, University of Kentucky, Lexington*

ABSTRACT

Varieties of United States tobaccos, tobacco introductions, and wild *Nicotiana* species were tested in the laboratory and in the field for resistance to the green peach aphid, *Myzus persicae* (Sulz.). Selections and crosses of resistant tobacco were less susceptible than any of the standard burley tobacco varieties. Ky. 61, TI 566B, and TI 567 were more susceptible than the other tobacco varieties tested. *Nicotiana gossei*, *N. repanda*, and *N. trigonophylla* were highly resistant, but crosses of *N. gossei* with *N. tabacum* did not show the high resistance of the *N. gossei* parent. The senescing leaves of the resistant wild species were much more susceptible to aphids than the young or mature leaves. The glucose content of *N. gossei* leaves was very low compared with that of leaves of susceptible plants, but from preliminary tests this relationship did not seem to be true for other resistant species.

Although the selection and breeding of various crop plants for resistance to insects has a long history, few experiments or observations have been published on resistance of tobacco to insects. Because of the successful breeding programs for disease-resistant tobacco varieties and the importance of tobacco as a crop, experiments were started in 1955 at the University of Kentucky on resistance in *Nicotiana* to the green peach aphid, *Myzus persicae* (Sulz.). Since that time other studies have been initiated on resistance in *Nicotiana* to other insect pests, such as hornworms, budworms, and flea beetles.

Since 1946 when the first heavy colonizations by the green peach aphid on tobacco in the United States occurred, heavy infestations have been found in all the major tobacco-producing areas of the United States. Shortly afterwards infestations also were found in Canada, the West Indies, and South America. The green peach aphid is not so important as some of the other insect pests of tobacco in the United States, but in certain areas it is always an important pest and in most other areas it is very destructive in individual fields. Because of its ability to transmit several tobacco disease organisms, the green peach aphid is potentially one of the most important tobacco insect pests.

METHODS AND MATERIALS.—Three approaches to the selection and breeding for resistance to the green peach aphid have been followed: (1) the use of wild *Nicotiana* species, (2) the screening of a large number of tobacco introductions (TIs) which have been gathered from most of the tobacco-producing areas of the world, and (3) the evaluation of resistance in some of the standard tobacco varieties and breeding lines being grown in the United States.

Field tests were usually run on plants grown in the manner typically used by tobacco farmers in Kentucky. In some of the tests the wild *Nicotiana* species were grown in 4-inch square peat moss pots in the greenhouse before transplanting rather than being grown in the plant beds. Plants were spaced 18 inches apart in the rows, and the rows were 42 inches apart. Where possible, plots were replicated in the same field and several ratings of aphid infestations were made during the growing season. Ratings of infestation were made on the upper leaves of the plants as follows: 0 = no aphids, 1 = very light infestation, 3 = moderate infestation, and 4 = heavy infestation. In some of the tests, plants were manually infested with apterous adults from natural field populations, but natural infestation by alate females was the preferred procedure and was used in most tests. Selections of individual plants for resistance or susceptibiltity were made on the basis of ratings throughout the season.

In the greenhouse tests infestations were produced by the manual transfer of apterous aphids, by introducing uninfested plants into greenhouses with populations of alate aphids or by the introduction of known numbers of alate females into large cages (4″×8″×4″) containing the test plants. It was easier to differentiate degrees of resistance in the TIs and in varieties of tobacco by the last method than it was by the first two procedures. Greenhouse cultures were replaced each fall from natural infestations on burley tobacco in surrounding fields.

For some of the selection work, leaf cages were used. These cages were made from the tops or bottoms of clear plastic petri dishes approximately 50 mm. in diameter and 15 mm. high. A hole 25 mm. in diameter was cut out and covered with fine muslin. A narrow strip of $\frac{3}{16}$-inch thick plastic foam was cemented to the rim of the cage. A stiff piece of cardboard was placed on the top of the leaf and the cage on the underside of the leaf. A thin strip of wood was placed over the cardboard and also over the top of the cage. Rubber bands held the ends of the pieces of wood together, forming a tight seal around the cage. No moisture condensation was formed inside the cages, and the aphids appeared to feed and reproduce normally.

RESISTANCE IN *Nicotiana*.—Of the 60 described species of *Nicotiana*, 40 were tested at least once in the greenhouse or in the field for resistance to the green peach aphid. Species from most sections of the genus and from the different geographical regions where *Nicotiana* species occur were tested. As expected, the range of resistance in the wild species was greater than in the TIs or in the United States varieties tested. *Nicotiana gossei*, *N. repanda*, and *N. trigonophylla* were the most resistant and *N. sylvestris* was the most susceptible of the species tested (table 1). When the plants were manually infested with second- or third-instar nymphs instead of by free-flying alate females, similar results were obtained (table 2). Wild species tested in the field were usually more resistant than when tested in the greenhouse. Under field conditions no infestations were ever found on *N. gossei* and *N. repanda*. Several species, such as *N. debneyi* and *N. goodspeedii*, which were susceptible in the greenhouse, were not infested by aphids in the field.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director. Accepted for publication March 28, 1961.

Selections of *N. gossei*, *N. repanda*, and *N. trigonophylla*, as well as other species with some resistance, were made because in all species there was some variability in resistance within the species. These selections of species and varieties are labelled R for resistant selections and G for susceptible selections. Crosses of 4n *N. tabacum* with *N. gossei* did not show resistance similar to that of the *N. gossei* parent. Although in some tests these crosses had smaller infestations than burley tobacco plants, the smaller size of the interspecific hybrids may have been responsible for this condition. Because of the lack of fertility of the pollen of this hybrid, not enough plants to test the inheritance of resistance have been available up to this time.

RESISTANCE IN *N. tabacum*.—More than 200 TIs, as well as many of the United States tobacco varieties, were screened in the field for resistance to the green peach aphid. TIs 764, 601, 532, 524, 555, 536, 494, and 497 were selected for resistance and TIs 566B and 567 for susceptibility. In the field in 1960 under conditions of natural infestations, selections of the resistant TIs, crosses of these TIs, and crosses and backcrosses with Kentucky 58 burley tobacco were evaluated (table 3). Two widely separated fields were used and, because of a lack of seed-

Table 1.—Susceptibility of *Nicotiana* species to the green peach aphid.[a]

Species of *Nicotiana*	Number of Aphids[b]				Susceptibility Rating[d]	
	Alates	Apterous				
	3/21/60	3/21	3/28	4/1[c]	Greenhouse	Field
acuminata					++++	
alata	12	62	300	1,350	++++	
benavidesii	15	45	322	2,000	++++	++++
benthamiana	3	10	17	500	++	++++
bigelovii	23	70	568	2,100	++++	
bonariensis	9	24	235	1,000	+++	
clevelandi	12	59	710	2,000	++++	
cordifolia					++++	
debneyi	2	19	197	1,100	++++	+
exigua	3	26	168	1,750	++++	
excelsior					++++	
glauca	6	28	99	550	+++	+++
glutinosa	20	36	342	1,550	++++	++++
goodspeedii	9	66	416	2,000	++++	+
gossei	5	18	26	325	+	+
knightiana	12	100	435	1,600	++++	++++
longiflora	12	21	165	1,100	++++	
maritima	1	3	101	650	+++	++
megalosiphon	9	10	107	500	+++	++
nesophila	1	7	70	400	+++	
nudicaulis	15	25	188	600	+++	++
occidentalis	16	63	264	750	+++	++++
otophora					++++	++++
palmeri					+++	
paniculata	17	68	520	1,350	++++	++++
pauciflora	14	38	137	1,100	++++	
plumbaginifolia	8	39	183	1,000	++++	
raimondi					++++	++++
repanda	7	5	9	400	+	+
rotundifolia	1	27	94	350	++	
rustica	21	134	785	2,250	++++	++++
setchelli					+++	
stocktonia	2		43	325	++	
suaveolens	16	65	620	2,500	++++	
sylvestris	61	51	444	2,250	++++	++++
tomentosiformis	22	50	235	1,500	++++	
trigonophylla	6	13	85	325	+	+
undulata	7	12	191	1,650	++++	
velutina	19	54	268	1,600	++++	
Ky 35	36	50	370	1,400	++++	++++
TI 566B	94	222	1,795	4,500	+++++	+++++
TI 567	160	341	1,960	4,500	+++++	+++++

[a] Infestations from alate females.
[b] Four plants of each entry placed in aphid-infested greenhouse on 3/11/60.
[c] Counts were estimates for this date rather than actual counts as on 3/21 and 3/28.
[d] Ratings for the greenhouse and field were often composites from several tests. Rating of + = highly resistant, ++ = resistant, +++ = slight to no resistance, ++++ = susceptible, and +++++ = highly susceptible.

Table 2.—Susceptibility of *Nicotiana* species and burley varieties when infested by green peach aphid nymphs.

Species of *Nicotiana*	Number of Aphids[a]				
	11/30	12/5	12/8	12/13	12/19
acuminata	18	47	71	247	556
alata	19	44	94	235	489
benthamiana	3	4	9	22	144
debneyi	18	36	57	203	363
excelsior	23	75	155	481	763
glauca	16	44	63	181	716
glutinosa	14	42	74	280	452
gossei	2	17	13	65	104
nudicaulis	22	60	77	244	556
occidentalis	16	51	156	320	839
palmeri	15	56	55	165	317
plumbaginafolia	17	79	105	383	607
repanda	3	6	4	67	184
repanda R	1	6	7	17	190
rustica	26	48	197	613	1,150
sylvestris	31	70	240	533	761
trigonophylla	4	9	15	27	144
trigonophylla R	9	9	8	31	111
undulata	24	57	134	304	799
velutina	11	33	56	191	962
Ky 9	23	81	186	468	1,271
Ky 16	13	43	104	347	787
By 21	27	67	168	379	777
Ky 35	12	63	117	279	667
Ky 41A	17	63	158	481	1,066
Ky 58	26	95	247	571	1,240
Ky 61	28	70	177	534	1,540

[a] Two second- or third-instar green peach aphids put on each of four plants per test species or variety on November 25, 1960.

Table 3.—Resistance to the green peach aphid in various lines of tobacco in the field. August 1960.

TIs and Burley	Number of Plants	Average Rating[a] per Plant		Infested Plants (%)
		All Plants	Infested Plants	
524×497	48	0.15	1.2	12
532×497	47	.10	1.0	11
494×497	48	.08	1.3	6
601×494	37	.24	1.5	16
601×536	37	.16	2.0	8
532×764	69	.09	1.0	9
764R[b]	49	.08	1.0	8
764×Ky 58	49	.33	1.3	24
(764×Ky 58)×Ky 58	48	.63	1.8	35
601×Ky 58	29	.34	1.7	21
(Ky 58×601)×Ky 58	38	.42	1.8	24
(536×764)×Ky 58	38	.21	2.0	11
555R	59	.34	1.5	22
(555×Ky 58)×Ky 58	37	.51	1.7	30
567G	69	2.74	2.9	94
Ky 9	38	.95	1.4	66
By 21	126	.90	1.6	58
Ky 35	40	.58	1.4	43
Ky 41A	40	1.23	1.7	73
Ky 58	27	.78	1.2	63
Ky 58R	51	.41	1.3	31
Ky 61	102	2.92	3.0	99
Ky 61G[c]	75	3.21	3.2	100
Ky 611[d]	30	3.30	3.3	100

[a] Rating of 0 to 4 with 0 = no aphids and 4 = heaviest population possible.
[b] R is selected for resistance and selfed.
[c] G is selected for susceptibility and selfed.
[d] I is irradiated seed.

Table 4.—Resistance of varieties and breeding lines of burley and dark tobaccos to the green peach aphid in three field tests.

Entries	Ratings of Aphid Resistance[a]				
	Test 1	Test 2		Test 3	
	Rating	Rating	Infested Plants (%)	Rating	Infested Plants (%)
Ky 151	0.5				
Black Mammoth	0.7				
Bird's Golden Burley	0.7				
Casen's Yellow Twist	0.8				
By 21		1.4	70	0.9	58
Ex 4		1.5	68		
GR 37		1.6	75		
Ky 33	0.8	2.5	95	0.6	43
Clarksville	0.9				
Ky 58R				0.4	31
Ky 58				0.8	63
By 1	0.9				
F1 (Ky 61×By 21)	0.9				
Rudolph's Mammoth	0.9				
N. debneyi sterile×By 21	1.0				
Ky 16	1.0	2.1	85		
Ky 9	1.0	2.0	80	1.0	66
Ky 41A		2.1	83	1.2	73
Ky 160	1.0				
Little Wood	1.0				
N. suaveolens×By 21	1.1				
N. debneyi sterile×Ex 1	1.1				
Va By 29	1.2				
F2 Ky 61×By 21	1.4				
F3 (Binehart×Ex 1)×3176	1.5				
GR 38		2.3	95		
Ex 1	1.5	2.9	95		
Ex 3		3.4	95		
TI 567 G	1.5			2.7	94
Ky 153	1.6				
Ky 61	1.6			3.0	99
Ky 61 G	1.7			3.2	100

[a] Ratings of Test 1 based on 0–3, and of Tests 2 and 3 on 0–4 with 0=no aphids.

lings for some selections, the number of replicates and number of plants were not the same for each entry. However, figures in this table are totals of at least three replicates with usually 10 plants per replicate. All the TIs selected for resistance were less heavily infested and had a lower percentage of infested plants than any of the standard burley varieties. Crosses and backcrosses of some of these TIs with Ky 58 were intermediate in resistance. Ky 58R was less heavily infested than Ky 58 or the other standard burley varieties. Ky 61, Ky 61G (a selection for susceptibility of Ky 61), Ky 61I (from irradiated Ky 61 seed), and TI 567 were the most susceptible lines with no significant differences among them. Selections of individual plants from the more promising lines are being evaluated in the greenhouse for further selection.

In field tests of burley and dark tobaccos infested naturally, Ky 61, Ky 153, Ex 1 (Experimental 1—a Kentucky breeding line), and TI 567 were highly susceptible (table 4). There was no indication that the dark

Table 5.—Infestations of mature *Nicotiana* species and tobacco varieties by alate green peach aphids.[a]

Entries	Date in 1959					
	1/19	1/27	1/31	2/4	2/10	2/16
N. gossei	0	0	4	5	6	91
N. trigonophylla	9	13	11	29	42	565
TI 566 B	79	128	455	2,460	8,500	26,000
Ky 58	25	7	57	220	580	2,500
Ky 61	32	7	50	290	1,460	7,600

[a] Totals of six replicates per treatment. Uninfested plants placed in infested greenhouse on January 14, 1959.

Table 6.—Composite table from two experiments on the effect of leaf position on survival and reproduction of the green peach aphid.[a]

Plant	Leaf Position	Number of Live Aphids on Days After Transferral Shown[b]			
		1	2	3	6
Gossei	Upper	3+0	0+0	0	0
	Middle	9+0	7+0	4+3	2+10[c]
	Lower	20+0	11+14	7+23	4+30
Burley 21	Upper and Lower	32+0	31+12	30+47	25+121

[a] Thirty-four nymphs total transferred to five cages on each leaf position.
[b] First figure is number of original aphids and second figure is number of aphids produced by original aphids.
[c] All aphids from one out of the five cages.

tobaccos were more susceptible in general than the burley tobaccos. In Test A there were two or three replicates with 10 plants per replicate; in Test B there were four replicates with 10 plants per replicate; and Test C is taken from table 3. Although By 21 has consistently rated less susceptible than most other standard burley varieties, fields of By 21 have been found with heavy aphid infestations. It would appear that no variety tested has enough resistance to be used without further selecting of individual burley plants. Ky 61 is so much more susceptible that, when grown commercially, this variety is often harmed economically by green peach aphid infestations when other varieties are not. Ex 1, from these tests and from observations of large scale field plantings, resembles Ky 61 in this respect.

When nearly mature plants of two resistant *Nicotiana* species, two susceptible TIs, and two burley varieties were placed in an infested greenhouse, the *Nicotiana* species were lightly infested and the susceptible TIs were heavily infested (table 5). Under field conditions Ky 61 will sometimes be more heavily infested than TI 566B or 567. The susceptible TIs, however, tend to have a more equal distribution of aphids from the bottom to the top leaves than Ky 61. Even when heavily infested, Ky 61 and other burley varieties have very few aphids on the lower or middle leaves compared with the top leaves, suckers, buds, flowers, and seed pods.

Nature of Resistance.—The nature of resistance in *Nicotiana* to the green peach aphid is not known. Observations and experiments on aphid behavior on resistant species, however, offer some suggestions. Aphids are rarely found on the upper leaves of highly resistant species, such as *N. gossei* and *N. repanda*. In the greenhouse under heavy aphid population pressure, fairly large infestations may be found on the lower, senescing leaves of these plants. This explains the large numbers of aphids found on these resistant plants on the last date when counts were made in tables 1 and 2. Some of the moderately resistant species do not show this pattern of distribution.

When large numbers of aphids of various instars were transferred to the top leaves of *N. gossei* plants, most, if not all, of these aphids died on these top leaves within 48 hours. When aphid nymphs were caged on upper (young), middle (mature), and lower (senescent) leaves of *N. gossei* plants, all of the aphids on the upper leaves died within 48 hours (table 6). Aphids on the middle leaves

died somewhat more slowly, while those on the lower leaves lived longer and in some cases reproduced and produced colonies. Upper leaves of *N. gossei* when cut off and placed with the stem in tap water supported colonies of aphids several days afterwards. Uncut leaves above and below the excised leaves did not support such infestations.

These tests and observations indicate either a lack of feeding, a nutritionally deficient diet, or the presence of some toxic substance in the sap of these young and mature leaves. The increase in longevity of aphids on the lower leaves indicates some change in the senescing leaves. The rapidity of death of aphids on the upper leaves does not suggest normal feeding on a nutritionally deficient diet. Aphids probed these leaves readily and injected their stylets into the tissue but whether they actually fed or not is not known. No honeydew was produced by these aphids.

In some tests, using a rapid method of analysis, glucose appeared to be much lower in the upper leaves than in the lower leaves of *N. gossei* plants or in leaves of standard burley varieties. *N. repanda* and *N. trigonophylla* plants in these tests also contained smaller amounts of glucose than susceptible plants. When more accurate analyses were run, however, only *N. gossei* plants showed consistently low levels of glucose. More work is being done on this with plants being grown in nutrient solution to obtain more uniform conditions of plant growth.

RESISTANCE TO OTHER INSECTS.—Work on resistance to tobacco budworms (*Heliothis* spp.) and tobacco hornworms (*Protoparce* spp.) has not progressed as far as the work with aphids. There was a wide range of feeding preference by these insects on the various *Nicotiana* species, but in cage tests with moths of *Heliothis zea* (Boddie) there were no such preferences in egg laying. In field and greenhouse tests, *N. repanda*, *N. exigua*, *N. trigonohpylla*, and *N. velutina* showed resistance to hornworm larvae. In similar tests, the first three species and *N. nudicaulis* showed some resistance to larvae of *Heliothis zea*. *N. tomentosiformis* was especially heavily attacked by hornworms, and *N. debneyi* appeared very attractive to several grasshopper species.

Budworms and hornworms ate leaves of all species offered when other food was not available. Long-term feeding tests were not carried out, so it is not known if there would be a difference in growth or survival rate if these insects were fed exclusively on some of the *Nicotiana* species.

A Laboratory Rearing Method and Dosage-Mortality Data on the Bollworm, *Heliothis zea*[1]

J. R. BRAZZEL,[2] HOWARD CHAMBERS,[3] and P. J. HAMMAN[3]

ABSTRACT

A method for rearing the bollworm, *Heliothis zea* (Boddie), on a larval diet was developed. After larvae fed for about 2 days on cotton terminals they were transferred to individual vials which contained larval diet. Antimicrobial agents were added to the diet to eliminate the necessity for aseptic procedures. This method proved satisfactory for rearing large numbers of bollworm larvae for laboratory testing.

Dosage-mortality curves were obtained for eight insecticides and insecticide combinations. The data obtained in this study indicate no measurable insecticide resistance in bollworms collected at College Station, Texas.

Reports of suspected resistance in the bollworm, *Heliothis zea* (Boddie), to currently effective insecticides have become quite frequent during the past 3 years in the Cotton Belt. In order to evaluate these bollworm populations for resistance there are needed reference dosage-mortality data for purposes of comparison. These reference data were obtained with a standardized rearing and testing method which enables the researcher to detect relatively low levels of resistance which might result in poor field control of the bollworm. A rearing procedure using an artificial diet and reference dosage-mortality data obtained in the College Station, Texas, Entomology Research Laboratory are herein reported.

Relatively little toxicological work has been done with the bollworm, which is a major pest of cotton in this State. Studies on the relative toxicity of some insecticides to the bollworm and the tobacco budworm, *Heliothis virescens* (F.), were reported by Brazzel et al. (1953) and Gast et al. (1956). The response of the various instars of the bollworm and tobacco budworm to insecticides applied topically were reported by McPherson et al. (1956) and Gast (1959). These data are available for comparison; however, Gast (1959) used field-collected larvae and McPherson et al. (1956) used larvae reared in the laboratory on various host plants.

In order to standardize the procedure and eliminate possible host plant effects on susceptibility, an artificial diet for rearing was found desirable. A diet suitable for this purpose was reported by George et al. (1960).

METHODS.—*Larval Rearing.*—The bollworm larvae used in the 1959 experiments were reared by the method described by Brazzel et al. (1953). Ten to twelve adults were confined in 1-gallon glass jars with honey diluted with water (1–1) for food. Eggs were laid on the cloth cover and cloth streamers hanging from the top of the jar. The cloth on which the eggs were deposited was removed from the oviposition cages at 2-day intervals and placed under glass bowls 6 inches in diameter and 3 inches deep. Fresh-cut cotton terminals were placed under the bowls on top of the eggs. As the larvae hatched they crawled up onto the cotton terminals to feed. It was necessary to transfer the larvae to fresh food at 1- to 2-day intervals until they reached the desired larval instar for testing.

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[2] Associate Professor, Texas Agricultural Experiment Station, College Station.

[3] Research Assistants, Texas Agricultural Experiment Station, College Station.

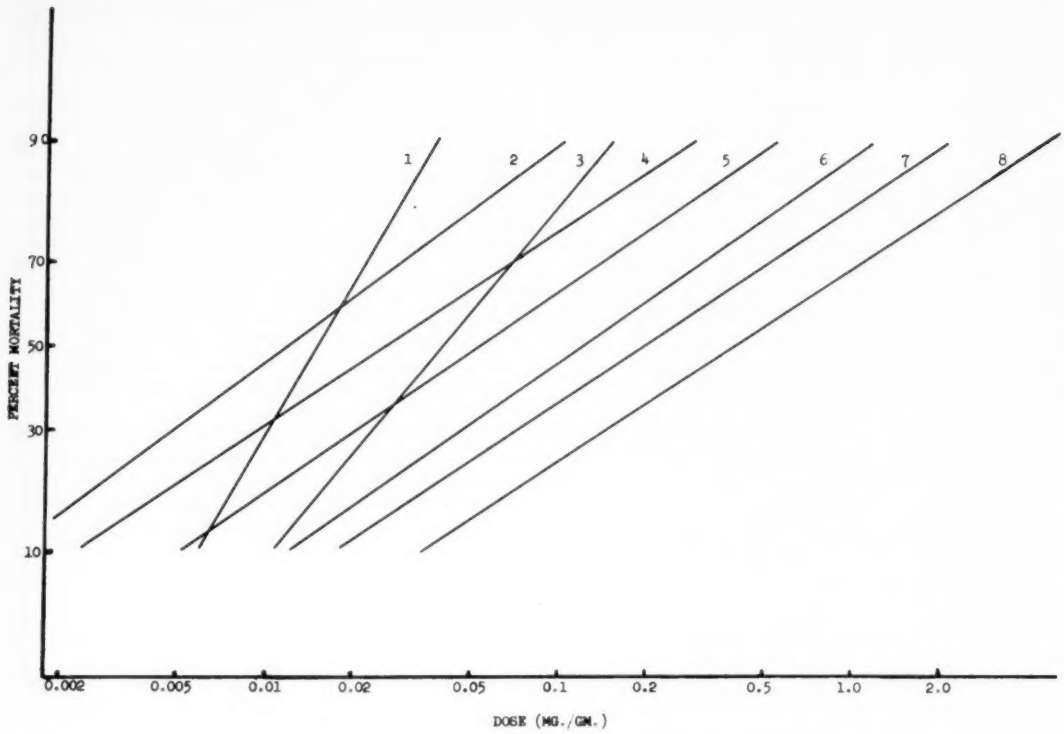

FIG. 1.—Dosage-mortality curves for the following insecticides and insecticide combinations expressed as milligrams of insecticide per gram of larval weight 48 hours after topical application: (1) Dilan, (2) Endrin, (3) Toxaphene-DDT, (2-1), (4) DDT, (5) Strobane-DDT (2-1), (6) Sevin, (7) Toxaphene, (8) Strobane.

As the larvae grew fewer numbers were placed in each bowl to eliminate loss by cannibalism.

All bollworm larvae used in the tests conducted in 1960 were reared in part on an artificial diet. The procedure for rearing through the first larval instar was the same as described for rearing in 1959 in the previous paragraph. The eggs hatched and larvae moved onto the cotton terminals and fed for about 2 days. It was necessary at this time to make the first transfer of the larvae to fresh food when reared by the method described above. At this time the larger and more vigorous larvae were transferred to cotton plugged shell vials containing the artificial diet, one larva per vial.

Transfer of the larvae to vials was accomplished with a No. 00 camel's-hair brush. It was found that it was difficult for the larvae to start feeding on the toughened surface of the solidified medium; but, they fed satisfactorily when the surface of the medium was scratched. Accordingly, after removal of the cotton plug from the vial, the surface of the medium was scratched with a hooked metal probe fastened to the upper end of the brush handle, the larvae were transferred and the cotton plug was replaced.

If the prepared vials were set upright, most of the larvae would crawl up to the cotton plug and starve. A rack was devised to hold the vials with the cotton plug down and the diet-filled end of the vial directed toward the light. This resulted in the larvae moving onto the diet and feeding normally.

Diet Preparation.—The diet reported by George et al. (1960) was used with minor modifications. A solution of antimicrobial agents to eliminate the necessity for aseptic procedure was added to the diet. This solution was prepared by mixing 20 grams of sorbic acid and 15 grams of methyl paraben in 170 milliliters of 95% ethyl alcohol (Brazzel et al. 1959). Also, 10% potassium hydroxide was added to adjust the pH to about 6.5.

The diet was mixed in a 1-quart Waring Blendor. The quantities of ingredients given by George et al. (1960) filled the mixer too full for efficient mixing. Accordingly, only one-half the amount of each ingredient was used to mix one batch of diet. To these ingredients were added 10 milliliters of the antimicrobial solution and 14 milliliters of 10% KOH.

The ingredients of the diet were mixed as follows:

(a) Weigh 11 grams of agar into container 1. Weigh 14 grams of alfalfa meal into container 2. Weigh 14 grams of Brewer's yeast, 17.5 grams of sucrose, 17.5 grams of casein, 5.5 grams of cellulose, 2.1 grams of Wesson salts, 1.4 grams of cholesterol and 0.2 gram of choline chloride into container 3.

(b) Add the agar in container 1 to 450 milliliters of

distilled water and heat until agar goes into solution. While the water and agar are heating, add 0.7 grams of corn oil+1% tocopherol, 10 milliliters of antimicrobial solution and 14 milliliters of 10% KOH to the mixing bowl of the 1-quart Waring Blendor. Add the water-agar solution and start the blender at slow speed. Add the ingredients in container 3 slowly to insure good mixing. Add the alfalfa meal in container 2 and mix at high speed for 1 to 2 minutes.

(c) The diet was poured into shell vials and allowed to solidify. Sterilized 4-dram shell vials 70 mm.×21 mm. in size with cotton plugs were used. The diet was poured to a depth of about one-half inch in each vial and the vials tilted to allow the diet to solidify in a slant. The prepared vials were held unplugged for about 2 hours to allow escape of moisture and than closed with cotton plugs. Bollworm larvae were placed on the diet within 24 hours after preparation.

Testing.—The bollworm larvae used in these tests were progeny of adults collected in a light trap at College Station with one exception, this being larvae tested with DDT in 1959 (table 1) which were the progeny of bollworms collected as sixth-instar larvae in a field in Burleson County in which bollworm resistance to DDT was suspected. The larvae used in all tests were in the third and fourth instar when treated.

In the 1959 tests larvae were removed from the cotton terminals furnished for food when they had reached the proper stage of development for testing, and were weighed individually. Scalar doses of DDT in one microliter of glass-distilled acetone were applied to the thoracic region of each larva. An acetone-treated control group was included in each test. Five doses of insecticide were applied to from 80 to 160 larvae at each dose. The treated larvae were held in groups of five in ½-pint jelly glasses with a fresh cotton terminal for food. Mortality records were made after 48 hours and the dosage computed as milligrams of insecticide per gram of larval weight.

In the tests conducted in 1960 the larvae were reared in individual containers. After development to the desired stage for testing, the larvae were removed from the vials one at a time, weighed, treated topically with one microliter of acetone solution of the insecticide and returned to the diet in the container. It was possible to make mortality records at 48 and 72 hours without removal of the larvae from the food. The larvae were reared and tested at a temperature of approximately 80° F.

Each insecticide was tested at three to five doses with 50 to more than 100 larvae tested at each dose. An acetone-treated control group was included in each series. The dosage for each group of larvae was computed in milligrams of insecticide per gram of larval weight. The LD_{50}'s were computed by probit analysis with fiducial limits at the 5% level.

Insecticides.—Insecticides used in this study included DDT, endrin, toxaphene, Strobane® (terpene polychlorinates, 65% chlorine), Dilan (2-nitro 1, 1-bis(*p*-chlorophenyl)propane and butane mixture, 1:2 ratio), and Sevin® (1-naphthyl *N*-methylcarbamate). Technical grade insecticides were dissolved in glass-distilled acetone to give the desired dose in 1 microliter of solution. The two insecticide combinations tested were mixtures of toxaphene and Strobane with DDT at a 2–1 ratio.

Table 1.—LD-50 values expressed as milligrams of insecticide per gram of larval weight for several insecticides 48 hours after they were applied topically to the bollworm.

Insecticide	No. Larvae Tested	Year Tested	Average Weight (mg.)	LD-50 (mg./g.)
DDT	480	1959	97.2	0.074± .0155
DDT	600	1959	74.7	.049± .011
DDT[a]	960	1959	99.3	.028± .0075
DDT	600	1959	177.0	.193± .0335
Endrin	285	1960	37.4	.012± .0045
Dilan	82	1960	42.7	.015± .006
DDT	394	1960	45.9	.026± .010
Toxaphene-DDT (2-1)	238	1960	42.9	.040± .0095
Strobane-DDT (2-1)	175	1960	46.4	.054± .028
Sevin	244	1960	45.2	.123± .047
Toxaphene	232	1960	39.1	.196± .0805
Strobane	189	1960	42.9	.419± .479

[a] Progeny of sixth-instar larvae collected from a cotton field in Burleson County in which bollworm resistance to DDT was suspected.

RESULTS AND DISCUSSION.—The rearing method for bollworms described herein using the diet reported by George et al. (1960) was excellent for rearing larvae from the second instar. From a practical standpoint of obtaining bollworm larvae for testing, there is little objection to transferring second instar larvae instead of eggs to the diet. The rearing of large numbers of larvae to the second instar described herein requires little effort. Also, the transfer of only the more vigorous second-instar larvae to the diet results in about 85% survival of larvae to the testing stage.

Sufficient diet was placed into each shell vial to rear a larva through the fourth instar. Thus, it was not necessary to handle the larvae until they were ready to test. The addition of the combination of antimicrobial agents used in the boll weevil (*Anthonomus grandis* Boheman) diet (Brazzel et al. 1959) did an excellent job of control of microorganisms. Since the larvae were held singly in shell vials, cannibalism was not a factor. The larvae reared in this manner were remarkably disease-free with less than 5% mortality attributable to diseases.

These results are in contrast to the rearing procedures using the host plants for food which require frequent food changing, and with high mortality usually from cannibalism and disease.

Some larvae were reared to pupation on the diet. In these instances it was necessary to place more diet in a larger vial or change the larvae to a fresh vial of diet during the fifth instar. Second instar larvae required from 5 to 7 days after transfer to the diet to reach the stage of development used in these tests.

The dosage-mortality curves for six insecticides and two insecticide combinations are presented in figure 1. These data were obtained in 1960 using the larval diet for rearing after the first instar of development.

The LD_{50} values for topical experiments in 1959 and 1960 are given in table 1. Included in this table are average larval weights of the test larvae and the number of larvae used to obtain the data on each insecticide.

These results indicate that endrin was the most effective with toxaphene and Strobane the least effective of the materials tested for bollworm control. Dilan appeared to be slightly less effective than endrin and more effective than DDT; however, this curve is based on relatively few data, table 1. DDT was the next most effective material

followed closely by the toxaphene-DDT (2–1) and Strobane DDT (2–1) combinations. Sevin was more effective than toxaphene and Strobane but less effective than the other materials.

All the dosage-mortality curves were relatively parallel with the exception of Dilan and toxaphene-DDT (2–1), which were steeper than the other lines.

The data presented in table 1 for tests in 1959 were obtained in an effort to determine if there was a difference in susceptibility in bollworms collected in a cotton field in Burleson County in which control difficulties had been encountered and bollworms collected in the College Station area. These results indicate no evidence of resistance to DDT in the bollworms collected in the field in question; in fact, these larvae were more susceptible to DDT than the larvae reared from adults collected in the light trap at College Station.

Two reports are available with data confirmed by portions of the data presented in this report. McPherson et al. (1956) obtained 67.7% control of bollworms ranging in weight from 64 to 132 milligrams with DDT doses ranging from 0.045 to 0.093 milligrams per gram of body weight. Gast (1959) obtained an LD_{50} of 0.040 milligrams of DDT per gram of body weight. In the DDT test in 1960 with larvae that averaged 45.9 milligrams in weight, 63% mortality was obtained with a dose of 0.021 milligrams per gram of body weight.

McPherson et al. (1956) obtained 62.5% control of bollworm larvae ranging from 58 to 103 milligrams in weight treated with endrin at 0.0060 to 0.0127 milligrams per gram of body weight. In the tests conducted in 1960 endrin applied at a rate of 0.0111 milligram per gram of body weight on larvae that averaged 37.4 milligrams in weight resulted in only 45% mortality. These results indicate less mortality on smaller larvae with endrin in 1960 than in 1954.

The data presented herein are primarily for reference purposes for comparison with later results in which insecticide resistance in the bollworm is suspected. It is a relatively simple matter to collect 25 to 50 sixth-instar larvae which have survived the insecticide programs in an area of suspected resistance, allow them to pupate and emerge as adults, rear their progeny as described herein (or by some comparable method), test and compare with these results.

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The Introduction and Establishment of *Dusmetia sangwani* on *Antonina graminis* in South Texas[1]

H. A. DEAN, MICHAEL F. SCHUSTER, and JACK C. BAILEY,[2] *Texas Agricultural Experiment Station, Weslaco*

ABSTRACT

An encyrtid parasite, *Dusmetia sangwani* Rao, was introduced from India during March and April 1959 and was found established on Rhodes-grass scale, *Antonina graminis* (Maskell), at 11 south Texas locations 16 months later. Some 1,136 parasites were screened through quarantine and 55,354 were reared in the laboratory and released at various locations. A mixture of royal jelly and honey was selected as a standard diet for laboratory propagation. Introduction shipments were provided the Republic of Mexico and the State of Arizona.

The most practical means of control of Rhodes-grass scale, *Antonina graminis* (Maskell), have been through the use of biological control, tolerant or resistant strains of grasses and cultural management. Field infestations of this scale insect have continued to increase during the spring and summer months owing to its reproductive potential and the lack of adequate biological control during this warmer season of the year. *Dusmetia sangwani* Rao was introduced during March and April 1959 in an effort to increase beneficial insect control of the scale.

An encyrtid parasite, *Anagyrus antoninae* Timberlake, had been introduced from Hawaii in 1948. The greatest extent of parasitism of this scale in the Lower Rio Grande Valley area was found during the October–March period, according to Dean & Schuster (1958). Attempts to establish the parasite on King Ranch were unsuccessful. A study of the available weather records showed the extended number of hours per day above 90° F. during the spring and summer months was associated with the failure of the parasite to become established on King Ranch. A very high percentage of parasitism was found beside a waterfall on a mountain side in Mexico, according to Dean (1960). The mountain shaded the location from the sun during the hottest period of the day, thus, the mild temperatures and continuous high relative humidity

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appeared to provide the best conditions for development of this parasite. It was desirable in south Texas to have a parasite which would not be limited in its activity by the high summer temperatures. Such a parasite might fill a gap in the parasite-control complex in the Lower Valley area and might offer a possible controlling agent for the areas of King Ranch.

Dusmetia sangwani was described by Rao (1957) from specimens reared from Rhodes-grass scale collected at Bangalore, India by H. S. Sangwan on March 4, 1957. The sexes are easily separated. The female is wingless and has a light tan thorax and a dark-colored abdomen, while the male is black and winged. The female has a white antennal club.

The Entomology Research Division of the U. S. Department of Agriculture made available parasitized Rhodes-grass scale purchased from the Government of India. The material was collected by B. R. Subba Rao from Rhodes grass at Bangalore and shipped to D. W. Jones of the Parasite Receiving Station at Moorestown, New Jersey. A total of 1,794 scales, presumably parasitized by *D. sangwani*, was isolated in individual one-half dram screw-cap vials and forwarded to Weslaco. Mr. L. W. Coles brought 565 by airplane and assisted in the early screening of the parasite material at Weslaco. Five shipments were made from the Parasite Receiving Station during the April 2–13 period. This material was handled in a quarantined room at Weslaco to avoid any possibility of utilizing or releasing parasites other than *sangwani*. The material produced 1,233 adult *sangwani* of which 97 were used to begin a laboratory culture. Details on the release of this material are given in table 1. The hyperparasite, *Cheiloneurus* sp., was removed in quarantine. Occasionally, one or two *sangwani* emerged from a scale from which hyperparasites emerged. However, it was a rarity for any *sangwani* to be produced from a scale which produced any *Cheiloneurus* parasites. Each parasitized scale produced from 1 to 30 *sangwani*.

Five shipments were made to Weslaco of adults which had emerged at Moorestown. Of the 114 adults received from Moorestown, only 58 were received alive at Weslaco, and many of these appeared to be in a very weakened condition. All adults were used in laboratory cage tests. These shipping tests indicated that this parasite was a "poor shipper" in the adult stage, as only 51% survived 2 days in transit.

LABORATORY PROPAGATION.—Individual cages were set up to gain early information which might be useful in laboratory propagation of the parasite. The root of the Johnson grass was planted in a vermiculite-peat-moss mixture. The stolon was drawn through a round hole in a milk bottle cap. Cellulose cotton was stuffed between the stolon next to the hole to prevent escape of the parasite. Both 4- and 12-inch lengths of $1\frac{5}{8}$-inch (inside diameter) glass tubing were fitted tightly over the inner ring of the bottle cap. The bottle cap which fitted over the other end of the tube had a $1\frac{1}{4}$-inch hole fitted with 52- ×52-mesh plastic screen. The screen allowed better circulation of air and less accumulated moisture to form on the side of the tube. Parasites were introduced into the cage with the scale-infested plant through the upper end of the tube.

Females, which emerged from a scale yielding only females, were placed in individual cages with Rhodes-grass scale on Johnson grass. These unfertilized *sagwani* females produced only male progeny.

The method of laboratory propagation of the parasites for field release was similar to a previously used method with several modifications. The rearing room was held at 75° to 78° F. and 65% to 75% relative humidity. Infested Johnson grass stolons were collected from the field and pruned to 4- to 8-inch lengths. The stolons were planted in vermiculite, a soil conditioner, which was placed in the bottom of the rearing cage. A framed cage covered with 52- ×52-mesh plastic screen had a door on one side. The number of parasites placed in each cage varied. The infested stolons were removed from the cage and placed in 1-gallon cylindrical cardboard food containers 2 to 5 days before emergence was expected. Parasites were collected from the containers each day, and the numbers recorded.

Parasites emerged in 25 to 35 days following the placing of the adults with the infested stolons. A ratio of 7 females to 1 male was found with the quarantined material while a slightly higher ratio was found in rearing records. However, a later paper will give a more accurate ratio number as well as more detailed information on the life history of the parasite.

Adults appeared to live for only a short time. Adults collected 1 day into 29- ×105-mm. lusteroid tubes were nearly all dead the following morning. The parasites were observed to consume a considerable amount of water. Parasites were thereafter given water in addition to either royal jelly, royal jelly plus honey, or honey. Droplets were placed on white nylon cloth placed over the ends of the tubes. Nylon cloth was an improvement over cotton cloth because of its poorer absorption property. However, honey would soak through the nylon cloth when large droplets were placed on the cloth or when smaller droplets were placed over an area where honey had been placed previously. The parasites would become trapped in the food which soaked through the cloth. No particular difference in mortality of the adults was noted with the various foods when the tubes were placed in an upright position. Less than 40% mortality was noted when the tubes were placed in a horizontal position. Tubes were placed in a horizontal position thereafter when tubes were left in the laboratory overnight and transported the following day to the field for release. Less mortality was noted with clear, 29- ×105-mm. lusteroid tubes than with the yellow, 32- ×162-mm. lusteroid tubes.

Supplemental foods were used possibly to increase the production of the parasite in the laboratory. The parasite

Table 1.—*Dusmetia sangwani* (screened through quarantine) released at various locations during April and May 1959.

LOCATION	HOST OF SCALE	NUMBER
Hoblitzelle Ranch, north of Mercedes	para grass	467
Wood's Airport, Donna	Rhodes grass	180
King Ranch, Norias	buffel grass	200
Ceasar, Kingsville	Rhodes grass	90
Headquarters	Rhodes grass	172
El Texano Ranch, Hidalgo	para grass	11
San Benito grass plots		16
	Total	1,136

Table 2.—*Dusmetia sangwani* (laboratory-reared) released at various locations during May 1959–October 1960 period.

LOCATION	ESTABLISHMENT BY AUGUST 1960[a]	PRINCIPAL GRASS HOSTS OF SCALE	NUMBER
King Ranch, Ceasar	*	Rhodes	1,506
Headquarters	*	Rhodes	3,799
Norias	*	Buffel, sandhill Rhodes	977 1,514
El Texano Ranch, Hidalgo	*	Para, Rhodes	3,319
Hoblitzelle Ranch	*	Para	2,878
Eddie Thomas Ranch, NW of Elsa		Buffel	830
Don Stone Ranch, Willamar		Rhodes	567
Delta Lake, Monte Alto	*	Para	2,471
Fort Brown, Brownsville	*	Johnson	881
Clifford Ranch, S of Mercedes		Rhodes	900
San Benito grass plots	*	Sorghum almum, Bermuda & others	579
Rio Grande City		Rhodes, Johnson	560
Winter Haven		Johnson, Bermuda	325
San Antonio		Bermuda, Johnson	442
Three Rivers		Bermuda, Johnson	300
Others—ranches, yards, canal banks, etc.		Bermuda, St. Augustine, Para, Johnson, Rhodes, etc.	33,506
		Total	55,354

[a] Positive recoveries indicated by asterisk.

had been observed to feed on the honeydew of the scale insect. Since the parasite was found to consume a considerable amount of water, water was given with all food supplements. The following food supplements were tested: A, royal jelly; B, 50% royal jelly plus 50% honey; C, royal jelly plus water; D, honey; and E, water alone. It became evident after nine tests that the diluted royal jelly offered no advantage over Diet A. Fewer parasites were produced and a fungus contaminant developed in the Diet C container. When the parasites were fed Diets A and B about the same number were produced with a 7.5 and 6.4 ratio of females to males. About the same number of parasites were produced when fed Diets D and E with a 9.1 and 7.6 ratio of females to males. Parasites fed Diets A and B produced 30% more progeny than parasites fed Diets D and E. Diet B was selected as a standard.

RECOVERY AND ESTABLISHMENT.—Recoveries of the parasite proved to be rather difficult from the scale on Para grass growing on the banks of lakes and canals. Numbers released seemed to be adequate, but for some reason, collected samples did not yield a recovery until the February-March period of 1960, several months after a release was made or after several releases were made. The Rhodes-grass scale parasite, *Anagyrus antoninae*, was present at these locations but its numbers appeared hardly adequate to be a factor in preventing establishment of *D. sangwani*. No particular difficulty was experienced with recovery of the parasite from scale on Rhodes or buffel grass at certain locations. No recovery attempt was made until 3 months following initial releases.

Establishment records are shown in table 2 where recovery was made in August 1960. Recovery was easily made in October 1959 at the three King Ranch locations and at Brownsville. Other establishment records have been from the scale on indicated hosts at the following locations: Johnson grass, just north of Mission; Para grass, canal bank in Mercedes; and Rhodes grass, Wood's Airport, Donna. It was interesting that no difficulty was experienced with recovery from the scale on Rhodes grass and buffel grass.

OTHER INTRODUCTIONS.—Introductory material was furnished Mexico and the State of Arizona. On September 25, 1959, four boxes of parasitized material were released to a biological control worker from Tampico, Mexico. Also, 255 female and 27 male *sangwani* were released for this introduction into Mexico. Only 10 females and 5 males were reported alive when the worker reached Tampico the following day. At least 2,000 parasites were estimated as the potential emergence from the quarantined material. Parasitized Rhodes-grass scale was also furnished Dr. George D. Butler, Jr., of the Department of Entomology, Tucson, Arizona. One hundred seventy-four parasitized scales, each isolated in individual one-half dram, screw-cap vials, were estimated possibly to yield more than 1,000 parasites.

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COMMON NAMES OF INSECTS

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By Jean L. Laffoon, Chairman, Committee on Common Names of Insects

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The Insecticidal Properties of Esters of Phosphonic and Phosphinic Acids[1]

T. R. Fukuto, R. L. Metcalf, and M. Y. Winton, *University of California, Riverside*

Abstract

A large number of new alkyl *p*-nitrophenyl ethylphosphonates and *p*-nitrophenyl dialkylphosphinates were synthesized and examined for toxicity to house flies, *Musca domestica* L., anticholinesterase activity, and stability to aqueous hydrolysis. The alkyl *p*-nitrophenyl ethylphosphonates maintained high insecticidal activity over a wide variety of alkyl groups. The phosphinate esters were slightly less toxic to house flies than analogous phosphonate esters but activity was still high. Both series of compounds gave rough linear log relationships between rates of inhibition and hydrolysis. Some of the compounds showed unusually high anticholinesterase activity and the influence of steric factor in heightening inhibition rates was demonstrated.

In earlier reports from this laboratory concerning the relationship of the chemical structure of a series of alkyl *p*-nitrophenyl alkylphosphonates with chemical reactivity and insect toxicity, the effect of changes in the alkyl group R in the structure shown below was examined (Fukuto & Metcalf 1959, Fukuto et al. 1959).

A preliminary report of the insecticidal activity of a few compounds in which R′ is varied from methyl to *n*-decyl and R is ethyl was also reported (Fukuto et al. 1959). Lengthening of the R′ chain seemed to have little effect on the toxicity of these compounds to the house fly, *Musca domestica* L., and only until R′ became *n*-decyl was a large decrease in toxicity noted. This series of compounds was expanded and examined further and the results are reported here. Also, since some of the most potent anticholinesterase agents known, such as soman (3,3-dimethyl-2 butyl methylphosphonofluoridate) and sarin (isopropyl methylphosphonofluoridate), are compounds in which the alkoxy alkyl is branched it was of interest to examine analogous compounds in which R′ in the structure above also contains branching.

In addition to phosphonic acid esters, a number of dialkylphosphinic acid esters of *p*-nitrophenol were prepared and examined. Compounds of this type have recently received considerable attention. Razumov et al. (1957) described a series of *p*-nitrophenyl and other substituted dialkylphosphinates of the general structure shown below.

R = methyl to *n*-butyl

Of these compounds *p*-nitrophenyl dipropylphosphinate was found to be as strong an inhibitor of cholinesterase as ethyl *p*-nitrophenyl ethylphosphonate, but 20 times less toxic to the white mouse. No insect toxicity data were reported, however. More recently, Schrader et al. (1959) in a patent application described the synthesis and insecticidal properties of about 150 new dialkylphosphinate analogs of known organophosphorus insecticides. The interest generated in these compounds prompts us to report our findings on the effect of changes in the dialkyl groups in a series of *p*-nitrophenyl dialkylphosphinates on insect toxicity and chemical reactivity.

Materials and Methods.—*Dialkyl Ethylphosphonates.*—Dialkyl ethylphosphonates were prepared from ethylphosphonic dichloride and the appropriate alcohol. The following procedure for the synthesis of dihexyl ethylphosphonate is typical. To a stirred mixture of 50 ml. *n*-hexyl alcohol, 27.2 ml. pyridine, and 100 ml. *n*-hexane was added slowly 25 g. ethylphosphonic dichloride. The mixture was stirred for several hours at room temperature and poured into water. The *n*-hexane layer was washed with dilute hydrochloric acid, dried over anhydrous sodium sulfate, and the product distilled, b.p. 100–115° C. (0.1 mm.). The physical constants of new dialkyl ethylphosphonates are given in table 1.

Alkyl Ethylphosphonochloridate.—The intermediate alkyl ethylphosphonochloridates were prepared as described previously by Fukuto & Metcalf (1959). These were not characterized but were converted directly into the corresponding alkyl *p*-nitrophenyl ethylphosphonates. The boiling points of the chloridates are given in table 2.

Alkyl p-Nitrophenyl Ethylphosphonates.—In all cases the alkyl *p*-nitrophenyl ethylphosphonates were prepared as described previously (Fukuto & Metcalf 1959) by the condensation of sodium *p*-nitrophenoxide and alkyl ethylphosphonochloridate. The physical constants of new alkyl *p*-nitrophenyl ethylphosphonates are given in table 2.

Dialkylphosphinic Acids.—Most of the dialkylphosphinic acids were prepared by known methods. Symmetrical dialkylphosphinic acids were prepared according to Kosolapoff (1949) by the reaction between *N,N*-diethyl-*P*-ethylphosphonamidic chloride and the Grignard reagent of the corresponding alkyl halide. In most cases the dialkylphosphinic acid was not isolated but converted directly into dialkylphosphinic chloride by the action of phosphorus pentachloride. Diethyl-, dipropyl-, di*iso*propyl-, and di-*n*-butylphosphinic chloride have been described by Razumov et al. (1957). Ethyl*iso*propylphosphinic acid has been described by Crofts & Fox (1958). Ethylphenylphosphinic acid, m.p. 79°–81° C. (Kosolapoff 1950) and diphenylphosphinic acid, m.p. 189°–190° C. (Kosolapoff 1942) were prepared according to Kosolapoff (1949). Ethyl-*t*-butylphosphinic acid was prepared according to the procedure described by Crofts & Fox

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Table 1.—Physical constants of new dialkyl ethylphosphonates

$$C_2H_5P(OR)_2$$
(O)

R	B.p.		n_D^{30}	Formula	Analyses	
	° C.	Mm.			Calculated	Found
n-C_5H_{11}	75–78	0.05	1.4288	$C_{12}H_{27}O_3P$	C 57.58 H 10.88	C 57.48 H 10.97
n-C_6H_{13}	110–115	0.1	1.4322	$C_{14}H_{31}O_3P$	C 60.40 H 11.21	C 60.93 H 11.76
n-C_7H_{15}	117–120	0.05	1.4352	$C_{16}H_{35}O_3P$	C 62.71 H 11.51	C 63.00 H 11.50
n-C_8H_{17}	130–132	0.05	1.4383	$C_{18}H_{39}O_3P$	C 64.63 H 11.75	C 64.40 H 11.85
n-C_9H_{19}	150	0.05	1.4408	$C_{20}H_{43}O_3P$	C 66.26 H 11.96	C 65.98 H 11.98
n-$C_{10}H_{21}$	170–172	0.05	1.4430	$C_{22}H_{47}O_3P$	C 67.64 H 12.15	C 68.35 H 13.12
i-C_4H_9	70–74	0.7	1.4187	$C_{10}H_{23}O_3P$	C 54.03 H 10.42	C 53.63 H 10.47
s-C_4H_9	62–68	0.7	1.4180	$C_{10}H_{23}O_3P$	C 54.03 H 10.42	C 53.31 H 10.78
i-C_5H_{11}	80–86	0.05	1.4252	$C_{12}H_{27}O_3P$	C 57.58 H 10.88	C 57.36 H 11.61
*neo*pentyl	70–72	0.1	1.4192	$C_{12}H_{27}O_3P$	C 57.58 H 10.88	C 56.94 H 11.49
3-methyl-2-butyl	72–76	0.1	1.4270	$C_{12}H_{27}O_3P$	C 57.58 H 10.88	C 57.12 H 11.45
4-methyl-2-pentyl	76–84	0.1	1.4262	$C_{14}H_{31}O_3P$	C 60.39 H 11.22	C 59.89 H 11.73
3,3-dimethylbutyl	94–100	0.2	1.4301	$C_{14}H_{31}O_3P$	C 60.39 H 11.22	C 59.20 H 11.67

(1958). The compound melted at 98°–99° C., elemental analysis: per cent calculated for $C_6H_{15}O_2P$, C 47.98, H 10.06; found, C 47.80, H 10.73.

Ethyl Ethyl-n-Propylphosphinate.—Because of the ready availability of ethyl ethylphosphonochloridate it was of interest to see if unsymmetrical phosphinates could be prepared by the direct action of the Grignard reagent on this compound. The Grignard reagent prepared from 7.42 g. magnesium and 52 g. propyl iodide was added dropwise to an ice-cooled solution of 43.4 g. ethyl ethylphosphonochloridate in 200 ml. ether. Mixture was allowed to stand overnight and decomposed with 150 ml. 2 M hydrochloric acid. The ether layer was separated, combined with chloroform extracts of the aqueous phase and dried over anhydrous sodium sulfate. Distillation gave 10 g. of ethyl ethylpropylphosphinate, b.p. 102–107° C. (10 mm.), n_D^{30} 1.4338, d_{30} 0.9604, elemental analysis: per cent calculated for $C_7H_{17}O_2P$, C 51.22, H 10.44; found, C 51.82, H 11.32.

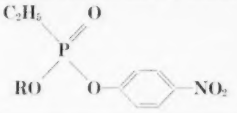
p-Nitrophenyl Dialkylphosphinates.—These compounds were prepared by the condensation of sodium *p*-nitrophenoxide and the dialkylphosphinic chloride in the usual manner. *p*-Nitrophenyl diethyl-, dipropyl-, di*iso*propyl-, and dibutylphosphinate have been described by Razumov et al. (1957). The physical constants of new *p*-nitrophenyl dialkylphosphinates are given in table 3.

Dipropyl and dibutyl *p*-nitrophenyl phosphate apparently have not been reported in the literature. These compounds were prepared in the customary manner from sodium *p*-nitrophenoxide and dipropyl or dibutyl phosphorochloridate in hot toluene. Dipropyl *p*-nitrophenyl phosphate distilled at 168°–170° C. (0.1 mm.), n_D^{30} 1.4977, elemental analysis: per cent calculated for $C_{12}H_{18}NO_6P$, C 47.52, H 5.98; found, C 47.64, H 6.35. Dibutyl *p*-nitrophenyl phosphate was distilled in the falling-film molecular still at 180° C. (0.1 mm.), n_D^{30} 1.4929, elemental analysis: per cent calculated for $C_{14}H_{22}NO_6P$, C 50.75, H 6.70; found, C 49.61, H 7.38.

The technique for determination of contact toxicity (LD_{50}) to the common house fly has been described (Metcalf & March 1953).

The methods used to determine first-order rates of hydrolysis ($K_{h.d.}$) in 1/15 M disodium hydrogen phos-

Table 2.—Physical constants of new alkyl *p*-nitrophenyl ethylphosphates



R	B.P. of Intermediate Alkyl Ethyl-Phosphono-Chloridate		B.P.		n_D^{30}	Formula	Analyses	
	° C.	Mm.	° C.	Mm.			Calculated	Found
n-C_5H_{11}	94–96	5.0	162–166	0.1	1.5089	$C_{13}H_{20}NO_5P$	C 51.82 H 6.69	C 51.34 H 6.79
n-C_6H_{13}	90–92	1.0	150[a]	0.1	1.5049	$C_{14}H_{22}NO_5P$	C 53.33 H 7.03	C 53.20 H 7.44
n-C_7H_{15}	98–100	0.5	160[a]	0.3	1.5027	$C_{15}H_{24}NO_5P$	C 54.71 H 7.35	C 54.63 H 7.76
n-C_8H_{17}	94–96	0.1	160[a]	0.1	1.5006	$C_{16}H_{26}NO_5P$	C 55.95 H 7.63	C 54.93 H 7.90
n-C_9H_{19}	115–118	0.7	180[a]	0.3	1.4996	$C_{17}H_{28}NO_5P$	C 57.13 H 7.90	C 57.21 H 8.05
n-$C_{10}H_{21}$	120–122	0.1	165[a]	0.08	1.4936	$C_{18}H_{30}NO_5P$	C 58.21 H 8.15	C 58.27 H 8.87
i-C_4H_9	82–86	5.5	140–144	0.1	1.5096	$C_{12}H_{18}NO_5P$	C 50.16 H 6.32	C 50.30 H 6.63
s-C_4H_9	72	2.5	140[a]	0.05	1.5090	$C_{12}H_{18}NO_5P$	C 50.16 H 6.32	C 49.40 H 7.06
i-C_5H_{11}	68–70	0.9	150	0.1	1.5080	$C_{13}H_{20}NO_5P$	C 51.82 H 6.69	C 51.58 H 7.22
*neo*pentyl	72–80	1	140–144	0.1	1.5056	$C_{13}H_{20}NO_5P$	C 51.82 H 6.69	C 51.95 H 7.68
3-methyl-2-butyl	68–72	0.3	145[a]	0.1	1.5060	$C_{13}H_{20}NO_5P$	C 51.82 H 6.69	C 51.36 H 7.37
4-methyl-2-pentyl	70–72	0.8	145[a]	0.05	1.5024	$C_{14}H_{22}NO_5P$	C 53.33 H 7.03	C 53.34 H 7.50
3,3-dimethylbutyl	60–62	0.1	150–154	0.1	1.5051	$C_{14}H_{22}NO_5P$	C 53.33 H 7.03	C 53.33 H 7.46

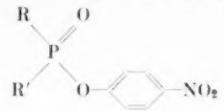
[a] Distilled in falling-film molecular still.

phate and second-order rates of reaction with fly-brain cholinesterase (K_c) have been described by Fukuto & Metcalf (1959).

The inhibition of fly-brain cholinesterase in the presence of acetylcholine was determined by the procedure described by Tammelin (1958). The instrument used was the Di-functional Recording Titrator manufactured by the International Instrument Company, Canyon, California. The activity of house fly-brain cholinesterase was followed by the consumption of 0.1 N sodium hydroxide in a reaction mixture consisting of 1.0 ml. 0.05 M acetylcholine bromide, 8.9 ml. of 0.1 M potassium chloride, and 0.1 ml. of an acetone solution containing the inhibitor. The reaction was carried out at 37.5° C. at pH 7.0. The inhibition of cholinesterase in the absence of acetylcholine differed in that lower concentrations of inhibitor were used and the inhibitor and homogenate were incubated for progressive time intervals before the addition of acetylcholine.

RESULTS AND DISCUSSION.—*Phosphonate Esters.*—In earlier work with a series of ethyl *p*-nitrophenyl alkylphosphonates (Fukuto & Metcalf 1959), it was found that the chemical reactivity and toxicity of these compounds to house flies was largely dependent on the nature of the alkyl moiety. Plots of log LD_{50} against log K_c (second order inhibition constant of fly-brain cholinesterase) for the various compounds showed that a linear relationship exists between these values. The relationship between log K_c and log $K_{hyd.}$ (first order hydrolysis constant in phosphate buffer) was not linear, but the overall trend was

Table 3.—Physical constants of new *p*-nitrophenyl dialkylphosphinates



R	R′	B.P. of Intermediate Dialkylphosphinic Chloride °C.	Mm.	B.P.[a] °C.	Mm.	n_D^{30}	Formula	Analyses Calculated	Found
C_2H_5	C_3H_7	98–100	4.5	140	0.1	1.5411	$C_{11}H_{16}NO_4P$	C 51.36 H 6.27	C 50.77 H 7.01
C_2H_5	C_4H_9	82	0.2	150	0.1	1.5351	$C_{12}H_{18}NO_4P$	C 53.12 H 6.70	C 53.93 H 7.00
C_2H_5	C_5H_{11}	100–102	1.0	160	0.1	1.5365	$C_{13}H_{20}NO_4P$	C 54.72 H 7.07	C 54.81 H 6.96
C_2H_5	C_6H_{13}	90–94	0.1	160	0.1	1.5241	$C_{14}H_{22}NO_4P$	C 56.18 H 7.40	C 56.14 H 7.33
C_2H_5	C_7H_{15}	120–122	1.0	165	0.1	1.5212	$C_{15}H_{24}NO_4P$	C 57.50 H 7.72	C 57.26 H 8.03
C_2H_5	C_8H_{17}	130–132	0.5	170	0.1	1.5200	$C_{16}H_{26}NO_4P$	C 58.69[b] H 8.01	
C_2H_5	*i*-C_3H_7	85–90	2.5	130	0.1	1.5384	$C_{11}H_{16}NO_4P$	C 51.36 H 6.27	C 50.56 H 6.46
C_2H_5	*t*-C_4H_9	74–76	1	145	0.2	1.5381	$C_{12}H_{18}NO_4P$	C 53.12 H 6.69	C 53.12 H 7.15
C_2H_5	C_6H_5			80–81[c]		—	$C_{14}H_{14}NO_4P$	C 57.74 H 4.85	C 57.62 H 4.78
C_6H_5	C_6H_5	150–154	0.1	120–125[c]		—	$C_{18}H_{14}NO_4P$	C 63.72 H 4.12	C 63.38 H 4.28

[a] Distilled in falling-film molecular still.
[b] Proper elemental analysis could not be obtained for this compound. However, it liberated the theoretical quantity of *p*-nitrophenol in phosphate buffer.
[c] Melting point.

that compounds with higher values for K_i were also higher in $K_{hyd.}$ Thus, it was possible to correlate and assess the effect of various alkyl groups on cholinesterase inhibition, alkaline hydrolysis, and insecticidal activity.

From data taken from table 4 for the series of alkyl *p*-nitrophenyl ethylphosphonates under investigation, plots of log K_i against log $K_{hyd.}$ gave a rough linear relationship (fig. 1), indicating that the rate of cholinesterase inhibition of these compounds is also a direct function of the reactivity of the molecule. Of notable exception is the high K_i value shown by the 3,3-dimethylbutyl compound (XVIII). The rate of enzyme inhibition was about 10-fold greater than expected from its hydrolysis constant. The structural similarity of this compound and the natural substrate acetylcholine suggests that the enhancement of inhibition is due to steric attraction in which this compound is brought into close proximity with the reactive centers before reacting with the enzyme. The K_i values of the *n*-amyl (V) through the *n*-nonyl (IX) esters were also high and peak activity was found with the *n*-heptyl (VII) ester. Since the $K_{hyd.}$ values for these compounds remain fairly constant, an increase in the rates of cholinesterase inactivation is entirely unexpected. The high K_i values suggest that a steric factor is also operating here. In order to lend support to this hypothesis, rates of inhibition of fly-brain cholinesterase by ethyl, *n*-octyl, and 3,3-dimethylbutyl *p*-nitrophenyl ethylphosphonates were determined in the presence of acetylcholine using an automatic recording titrator at constant pH (Tammelin 1958). It is well known that acetylcholine will prevent the phosphorylation of cholinesterase at low concentrations of organophosphorus compound. In fact, it is this phenomenon which allows us to determine second-order rates of inhibition (Aldridge 1950). However, by increasing the concentration of inhibitor it is possible to establish a threshold concentration where acetylcholine no longer demonstrates this protective action and a measurable rate of reaction between cholinesterase and inhibitor is observed. By determining the loss of cholinesterase by phosphorylation in the presence of acetylcholine it is possible to obtain some order of the competition between phosphorus compound and acetylcholine with cholinesterase. The rates of inhibition of cholinesterase in the presence and absence of acetylcholine are given in table 5. The discrepancy in K_i (acetylcholine absent) found manometrically and by the automatic titrator is probably

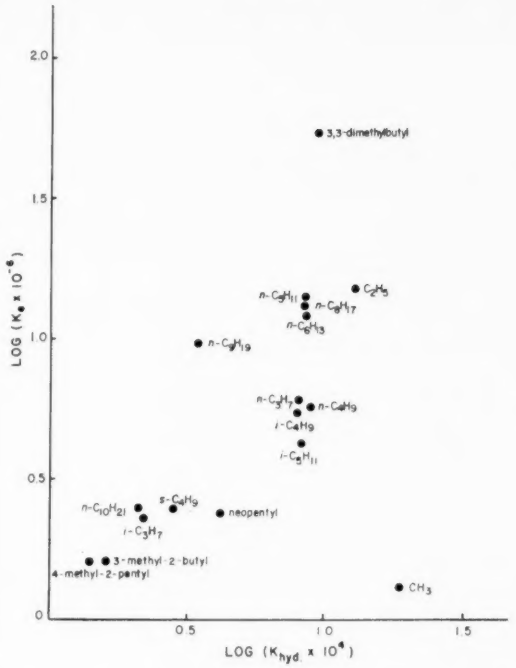


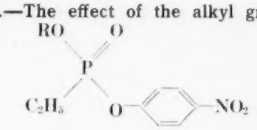
FIG. 1.—A plot of log cholinesterase inhibition constant K_e versus log hydrolysis constant K_{hyd} for alkyl p-nitrophenyl ethylphosphonates

due to the difference in reacting media. The K_e/K_e' ratios indicate that the n-octyl and 3,3-dimethylbutyl esters compete more strongly with acetylcholine than the ethyl ester. Since, according to their $K_{hyd.}$ values, the n-octyl and 3,3-dimethylbutyl esters are less reactive compounds it would appear that their high anticholinesterase activity is due partly to a competitive type mechanism in which steric factors are in operation.

The K_e value for the methyl ester (I) was lower than expected and is probably due to the high instability of this compound, i.e., it may decompose before reacting with the enzyme or the phosphorylated enzyme may spontaneously hydrolyze back to its active form. Esters of secondary alcohols (XI, XIII, XVI, and XVII) were generally lower in K_e but the values were consistent with their hydrolysis constants. Enhancement of inhibition by steric attraction by the 4-methyl-2-pentyl ester (XVII) was expected but none was found. 4-Methyl-2-pentyl methylphosphonofluoridate, a compound structurally analogous to XVII, is one of the most potent anticholinesterase agents known (Roeder & Kennedy 1955).

Plots of log K_e against log LD_{50} resulted in a wide scattering of points. Most of the compounds in table 4 were highly toxic to house flies in spite of widely varying K_e values. The most potent compound was the i-butyl ester (XII) with the methyl (I), ethyl (II), n-butyl (IV), and isopropyl (XI) esters almost as toxic. The K_e values (l./moles min.) for these compounds ranged from 1.5×10^7 for II down to 1.3×10^6 for I. The lack of correlation between toxicity and reactivity parameters is illustrated by the close K_e and $K_{hyd.}$ values of the n-decyl ester (X) and isopropyl ester (XI) compared to the 80-fold difference in toxicity.

Table 4.—The effect of the alkyl group R in



on toxicity, anticholinesterase activity, and rate of hydrolysis.

	R	$K_e\times10^{-6}$ (L. MOLES^{-1} MIN.$^{-1}$)	$K_{hyd.}\times10^4$ (MIN.$^{-1}$)	LD_{50} *Musca* (μG./G. FLY)
I	CH_3	1.3	18.9	1.2
II	C_2H_5	15.0	12.8	1.2
III	n-C_3H_7	6.0	8.15	1.4
IV	n-C_4H_9	5.7	8.78	1.5
V	n-C_5H_{11}	14.0	8.50	3.6
VI	n-C_6H_{13}	12.0	8.63	5.4
VII	n-C_7H_{15}	19.0	8.54	13.0
VIII	n-C_8H_{17}	13.0	8.42	14.5
IX	n-C_9H_{19}	9.6	3.50	55.0
X	n-$C_{10}H_{21}$	2.5	2.11	120.0
XI	i-C_3H_7	2.3	2.22	1.5
XII	i-C_4H_9	5.4	7.99	1.1
XIII	s-C_4H_9	2.5	2.83	3.1
XIV	i-C_5H_{11}	4.2	8.29	2.1
XV	*neo*pentyl	2.4	4.22	2.0
XVI	3-methyl-2-butyl	1.6	1.60	7.5
XVII	4-methyl-2-pentyl	1.6	1.49	16.5
XVIII	3,3-dimethylbutyl	54.0	9.44	2.7

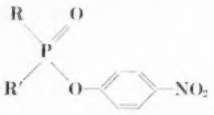
Phosphinate Esters.—The striking difference between alkyl alkylphosphonic acid esters of p-nitrophenol and an analogous series of dialkylphosphinic acid esters is the higher rates of hydrolysis and lower rates of fly-brain cholinesterase inactivation by the latter. Examination of the data (tables 4 and 6) for analogous phosphonate and phosphinate esters in which one of the alkyl groups is kept at ethyl and the other alkyl is varied from ethyl to n-octyl shows that the K_e values of the phosphinates are 5.4 to 29 times lower than the K_e values of the phosphonates. In the same series the $K_{hyd.}$ values for the phosphinates are 12.5 to 18.5 times greater than the corresponding values for the phosphonates.

It is apparent from these and other data (Fukuto et al.

Table 5.—Rates of inhibition of fly-brain cholinesterase in the presence and absence of acetylcholine.

COMPOUND	K_e (ACETYLCHOLINE ABSENT)	K_e' (ACETYLCHOLINE PRESENT)	K_e/K_e'
Ethyl p-nitrophenyl ethylphosphonate	5.7×10^6	6.1×10^4	93
n-Octyl p-nitrophenyl ethylphosphonate	3.9×10^7	2.1×10^6	19
3,3-Dimethylbutyl p-nitrophenyl ethylphosphonate	2.7×10^7	1.7×10^6	16
p-Nitrophenyl diethylphosphinate	5.1×10^5	3.9×10^3	131
p-Nitrophenyl ethyl-n-octylphosphinate	2.0×10^5	7.2×10^4	2.8

Table 6.—The effect of the alkyl groups R and R′ in



on toxicity, anticholinesterase activity, and rate of hydrolysis.

	R	R′	$K_e \times 10^{-5}$ (L. MOLES^{-1} MIN.$^{-1}$)	$K_{hyd.} \times 10^3$ (MIN.$^{-1}$)	LD_{50} (μG./G. FLY)
XIX	C_2H_5	C_2H_5	28	18.7	3.2
XX	C_2H_5	n-C_3H_7	2.1	15.1	3.7
XXI	C_2H_5	n-C_4H_9	5.5	14.3	4.9
XXII	C_2H_5	n-C_5H_{11}	7.5	11.9	10.5
XXIII	C_2H_5	n-C_6H_{13}	16	10.9	20.5
XXIV	C_2H_5	n-C_7H_{15}	21	11.2	125
XXV	C_2H_5	n-C_8H_{17}	13	10.8	>500
XXVI	C_2H_5	i-C_3H_7	4.3	3.59	6.5
XXVII	C_2H_5	t-C_4H_9	0.17	4.70×10^{-4}	22.0
XXVIII	C_2H_5	C_6H_5	79	64.0	16.5
XXIX	n-C_3H_7	n-C_3H_7	7.0	15.1	5.0
XXX	i-C_3H_7	i-C_3H_7	0.40	1.03	65.0
XXXI	n-C_4H_9	n-C_4H_9	1.4	1.33	500
XXXII	C_6H_5	C_6H_5	0.17	106	>500

1959) that the order of stability to aqueous hydrolysis of a homologous series of organophosphorus esters is phosphate > phosphonate > phosphinate. For example, the $K_{hyd.}$ (min.$^{-1}$) values of diethyl p-nitrophenylphosphate,

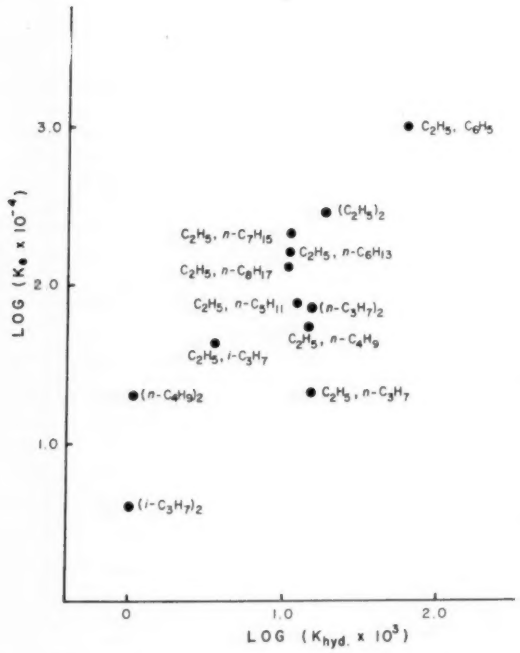
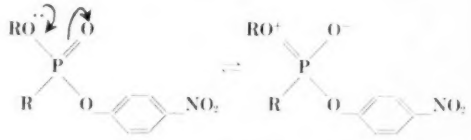
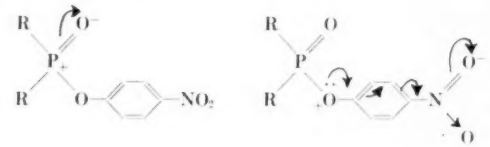


FIG. 2.—A plot of log cholinesterase inhibition constant K_e versus log hydrolysis constant K_{hyd} for p-nitrophenyl dialkylphosphinates

ethyl p-nitrophenyl ethylphosphonate, and p-nitrophenyl diethylphosphinate in 1/15 M disodium hydrogen phosphate are 6.14×10^{-5}, 1.20×10^{-3}, and 1.87×10^{-2}, respectively. On the basis of electronegativity differences alone between carbon, oxygen, and phosphorus, the reverse order is expected since the compound with the most electrophilic phosphorus atom would be most susceptible to hydrolytic splitting of the P-O-p-nitrophenyl bond. However, Heath (1956) points out that the electrophilic center of the phosphorus atom in the system RO—P=O is altered by the overlap of p-electrons of the alkoxy oxygen atom and the electrons in the P=O bond. The overall effect is that electrons are donated to phosphorus in the manner shown below, thus decreasing the electrophilic character of the phosphorus atom.



As the phosphorus atom can accommodate more than eight valency electrons (Phillips et al. 1945, Rothstein 1953) the above scheme is reasonable. The highly electrophilic center produced by the resonance structures shown below in a p-nitrophenyl dialkylphosphinate is not weakened by contribution of electrons from the weakly electron donating alkyl groups.



Thus, diethyl p-nitrophenyl phosphate with two ethoxy groups is expected to be more stable to hydrolysis than the corresponding phosphonate, which in turn is expected to be more stable than the phosphinate.

The inhibition of cholinesterase by the phosphinate esters in table 6 follows the same pattern as the phosphonates in table 4. A plot of log K_e against log $K_{hyd.}$ also gave a rough linear relationship (fig. 2). As with the phosphonate esters a notable rise in K_e occurred when the alkyl chain was lengthened from n-amyl (XXII) to n-octyl (XXV) and where the other alkyl group was kept at ethyl. Maximum activity was found with the n-heptyl compound (XXIV) That the same steric forces influence the activity of phosphinate esters is revealed also by the relative rates of inhibition of fly-brain cholinesterase by p-nitrophenyl diethylphosphinate and p-nitrophenyl ethyl-n-octylphosphinate in the absence (K_e) and presence (K_e') of acetylcholine (table 5). As discussed earlier with phosphonate esters the K_e/K_e' ratio of the long chain ethyl-n-octylphosphinate indicates that it competes more favorably with acetylcholine than does the diethylphosphinate. Branching, particularly on the one carbon atom, greatly lowered both K_e and $K_{hyd.}$ as exemplified by di*iso*propyl- (XXX), ethyl*iso*propyl- (XXVI), and ethyl-t-butylphosphinate esters (XXVII). The ethylphenylphosphinate (XXVIII) was the most potent inhibitor of

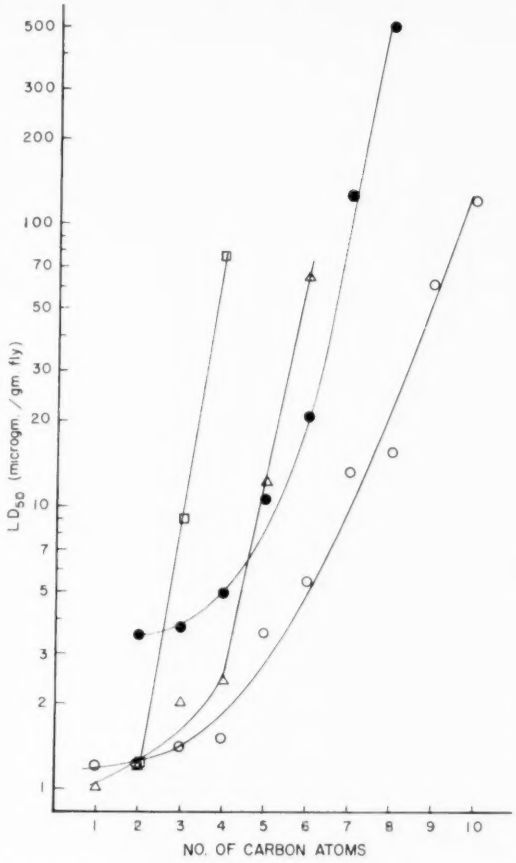
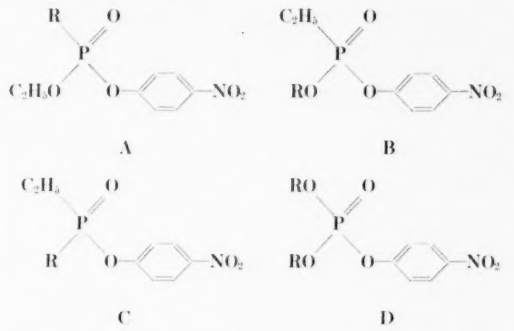


FIG. 3.—The relationship between toxicity to the common house fly and the number of carbon atoms in the straight chain alkyl groups for dialkyl *p*-nitrophenyl phosphates (□), ethyl *p*-nitrophenyl alkylphosphonates (△), alkyl *p*-nitrophenyl ethylphosphonates (○), and *p*-nitrophenyl ethylalkylphosphinates (●).

the series and is also one of the most reactive according to its hydrolysis constant.

Plots of log K_c against log LD_{50} for the phosphinate esters also gave a wide scattering of points. In general, these compounds were lower in toxicity to house flies than the analogous phosphonate esters and the values for LD_{50} are reflected in their lower K_c values. In comparing the toxicities of phosphinate and phosphonate esters containing the same alkyl groups it can be seen that for the most active compounds the former is about 0.33 times as toxic as the latter. The activity of the ethyl-*t*-butylphosphinate ester (XXVII) is exceptional in that it is considerably more toxic to house flies than ethyl *p*-nitrophenyl *t*-butylphosphonate ($LD_{50} > 5000$, Fukuto & Metcalf 1959). That XXVII should show any toxicity or inhibition of cholinesterase is difficult to explain in view of its extreme stability to aqueous hydrolysis.

The data in tables 4 and 6 show for the homologous series of phosphinate and phosphonate esters, in which one of the alkyl groups is kept at ethyl and the other group is a straight chain alkyl, that there is a gradual drop in insecticidal activity as the number of carbon atoms is increased. The relationship between house fly toxicity and number of carbon atoms is shown in figure 3 for the following series of compounds in which the number of carbon atoms in R is increased one at a time.



The data for the A series and for diethyl *p*-nitrophenyl phosphate were taken from Fukuto & Metcalf (1959). The values for LD_{50}, $K_{hyd.}$, and K_c for dipropyl *p*-nitrophenyl phosphate are 9.0 μg/g. fly, 6.40×10^{-5} min.$^{-1}$, and 1.0×10^6 l./mole min., respectively. The corresponding values, in the same units, for dibutyl *p*-nitrophenyl phosphate are 75.0, 6.1×10^{-5}, 9.9×10^5. It is apparent from figure 3 that phosphonate esters of type B, in which the alkoxy alkyl chain is lengthened, retain high insecticidal activity over a larger range of carbon atoms. Type A phosphonates showed the highest activity among the lower chain alkyls but toxicity dropped rapidly after the C_4 compound. The phosphinates were generally lower in toxicity and activity dropped at a rate in between the two phosphonate series. The toxicities of diethyl, dipropyl, and dibutyl *p*-nitrophenyl phosphate are also included and although a direct comparison with the other series cannot be made since the carbon atoms in both alkyls are increased it is interesting to note that a linear relationship in log LD_{50} and chain length is obtained.

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Pupal Mortality of the Orange-Striped Oakworm[1]

STEPHEN W. HITCHCOCK, *The Connecticut Agricultural Experiment Station, New Haven*

ABSTRACT

Less than half of the overwintering pupae of the orange-striped oakworm (*Anisota senatoria* (J. E. Smith)) produced adults in each of 2 years. The rate and causes of overwintering mortality varied from place to place within the study areas. Survival of overwintering pupae varied from 9% to 100%. *Cratichneumon w-album* (Cress.), reported from this host for the first time, parasitized about 10% of the pupae each year. Disease (*Cordyceps militaris* (Fr.) Link and an apparent virus) killed about 14% of the pupae each year. The remaining mortality was caused principally by *Winthemia datanae* (Twnsnd.). The effectiveness of *W. datanae* declined the second year of the study from a probable 42% to 16%.

The orange-striped oakworm (*Anisota senatoria* (J. E. Smith)) is a common lepidopterous pest of eastern woodlands that occasionally reaches outbreak numbers. Although its general life history is well known, the irregular nature of its abundance has apparently precluded the necessity for any exhaustive study of its biology. During the course of surveying damage caused by this insect, I made some observations on overwintering mortality which may serve to indicate some of the reasons for its decline in numbers following the outbreak year of 1958. Felt (1905), Thompson (1944), and Raizenne (1952) listed many natural enemies of this insect, but apparently little work has been done on the effectiveness of these parasites. Ordinarily, natural controls hold this oakworm in check although certain areas in the United States and Canada seem to be particularly liable to persistent infestations.

In Connecticut the orange-striped oakworm has only one generation a year and overwinters in the ground as a pupa from September or October to early July of the following year. Only a few parasites were of importance in attacking the overwintering stage during 1959 and 1960.

METHODS.—In each of 2 years, pupal counts were made spring and fall at 11 different locations scattered throughout the infested area. For these counts a square yard of forest floor was marked out and all litter in it was removed and examined. The soil beneath was then gradually scraped away and examined to a depth of 1½ inches. Six such square-yard samples were taken at each location. Broken or dead pupae were recorded and all other pupae were brought back to the laboratory to await the emergence of an adult moth or pupal parasite.

Table 1.—Pupal populations and survival of the orange-striped oakworm.

LOCATION	NO. OF PUPAE IN 6 SQUARE YARDS			PERCENTAGE OF MOTH EMERGENCE FROM SPRING PUPAE	
	Fall 1958	Spring 1959	Fall 1959	1959	1960
South Windsor	53	44	1[a]	36.4	100.0
Glastonbury	36	40	[a]	20.0	—
Lebanon	12	13	11	38.4	46.7
Plainfield	52	46	—	32.6	—
Norwich	67	34	3	8.8	25.0
Voluntown	53	47	5	14.9	42.9

[a] Treated with DDT in summer of 1959.

RESULTS.—Pupal counts from the most heavily infested plots are summarized in table 1. There was little overwintering loss in numbers of pupae during the winter of 1958–59 except at Norwich. Because of sampling variation, the differences between the spring and fall counts at Norwich were not significant. However, numerous broken and scattered pupal cases on the surface of the ground in the Norwich plot suggested that mammalian predators were the probable cause of some of this reduction. These broken pupal cases are not represented in table 2. The percentage of adult emergence from the spring pupae in 1959 varied from 8.8% to 38.4% and averaged 24.1% statewide. In 1960, however, the percentage of emergence increased about threefold in the study areas, except at Lebanon, and 44.8% of all pupae produced moths. An analysis of the causes of pupal mortality is given in table 2.

It was found that *Cratichneumon w-album* (Cress.) (det. P. B. Dowden and L. M. Walkley) remained overwinter in the pupal case of the oakworm and emerged the following spring, leaving a characteristic round emergence hole at the head end of the host pupa. Only one parasite emerged from each host pupa. Adults of this parasite were numerous by June 1 and were seen quartering over the

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Table 2.—Causes of death of overwintering pupae of the orange-striped oakworm in spring, 1959.

LOCATION	AVERAGE NUMBER OF PUPAE PER SQUARE YARD				
	Alive	Parasitized[a]	Parasitized[b]	Diseased[c]	Other
South Windsor	2.7	1.5	2.8	0.2	0.2
Glastonbury	1.3	0.3	3.0	0.5	1.5
Lebanon	0.8	0.2	0.5	0.5	0.2
Plainfield	2.5	0.8	2.7	0.7	1.0
Norwich	0.5	0.2	3.3	1.2	0.5
Voluntown	1.2	0.5	3.8	1.8	0.5

[a] *Cratichneumon w-album.*
[b] Definite and probable *Winthemia datanae.*
[c] "Virus" and recognizable *Cordyceps.*

ground a few inches above the leaf litter. However, an oakworm pupa was found as late as July 13 that contained a *Cratichneumon*. The last live adult was seen on September 25.

Parasitism by *Cratichneumon w-album* had no relation to the intensity of the infestation at various points in the State. It was heaviest at South Windsor and lightest at Norwich (table 2), both areas having had heavy infestations the previous summer. The rate of pupal parasitism by this insect remained relatively constant in 1959 and 1960, being 9.6% and 10.4%, respectively, even though the total numbers of available host pupae decreased. There was some slight evidence that *Cratichneumon* was more apt to be found in a female pupa than in a male.

Specimens of *Winthemia* nr. *datanae* (Twnsnd.) (det. W. Downes) were extremely numerous at South Windsor by September 3 in 1958 and were depositing many eggs. Likewise in 1960 *W. datanae* (det. C. Sabrosky) was plentiful by September 9 at Moosup. These flies took a heavy toll in the fall of 3 years. The female deposited her eggs on fully grown oakworm larvae just prior to oakworm pupation. When several female flies oviposited on the same larva, up to 138 eggs were found on a single oakworm larva. The full grown maggots left the body of the host and overwintered in the soil. From one to four maggots emerged from each pupa through the intersegmental membranes of the abdomen of the host, sometimes breaking the abdomen in two. A fly, presumably this species, was observed on July 21 attempting to oviposit on first and second instar oakworm larvae at South Windsor. No egg was actually deposited.

Most of the specimens listed under *Winthemia datanae* (table 2) were broken pupal cases which were probably caused by this insect, but may have been caused in small part by predators. In the absence of animal tunnels through the soil, or teeth marks on the pupal cases, it was considered that they were caused by this Tachinid parasite. This was especially indicated where adult flies were observed to be numerous in the fall. As it was difficult to assess accurately the control exerted by *Winthemia*, counts were made in the fall of 1959 of oviposition by *Winthemia* on 100 or more last instar oakworm larvae crawling over the ground. In an area of light defoliation and declining populations at Voluntown, 67.6% of the caterpillars had been attacked by *Winthemia*. At Lebanon, where the pest population remained approximately stationary, *Winthemia* had oviposited on 47% of the larvae. At Moosup in an area of heavy host density and complete defoliation only 25% of the caterpillars were attacked.

Evidences of two different diseases attacking the pupae were found in the study plots. The more common was *Cordyceps militaris* (Fr.) Link (det. E. A. Steinhaus). The other, which appeared irregularly in the study areas, was similar in gross morphological symptoms to the diseased condition associated with polyhedral bodies by Wallis (1959). This latter disease affected the greater number of pupae at Voluntown whereas *Cordyceps* was more prevalant elsewhere. The apparent virus and recognizable *Cordyceps* killed 13.6% of all pupae collected in 1959, and 14.9% of those collected in 1960. In the spring other pupae were found which were filled with a mass of fungous hyphae. These could represent undeveloped *Cordyceps* or saprophytic fungi in pupae killed by cold. As they were not numerous, it was considered that cold weather played little part in killing the overwintering pupae.

Small mammals fed on the overwintering pupae that remained in the ground from September until late June or July. Although this activity was not observed in the field, broken pupal cases with indications of chewing on them were found on the surface of the ground in several cases. In the laboratory a deermouse, *Peromyscus* sp., fed freely on the pupae.

DISCUSSION.—In Connecticut the orange-striped oakworm is ordinarily found only as a defoliator of an occasional roadside tree. Although these small centers of defoliation may persist for some time, only rarely do they coalesce into an area greater than a few branches or trees. Unfortunately, nothing is known of the reasons for the unprecedented outbreak in the summers of 1957 and 1958 when about 37,000 acres were defoliated by this insect. This outbreak was characterized by a sudden rise in the oakworm population, followed by 1 or 2 years of complete defoliation and then a rapid and irreversible decline over 2 years to the customary low level of population. The infestation moved generally from the vicinity of the Connecticut River eastward, but by the time defoliation had appeared in the eastern part of the State, the early infestations had already started to decline. A few spots persisted throughout the entire period of the infestation.

Cratichneumon w-album, reported here for the first time from the orange-striped oakworm, attacked the same proportionate number of hosts in both years studied even though the host population declined. Heinrich (1960) stated that some *Cratichneumon* species have two generations a year and this may also be so with *C. w-album*. In the event that this parasite oviposits only on fresh pupae as does *C. nigritarius* F. (Heinrich 1960), it would then need a second host species because the orange-striped oakworm has only one generation a year in Connecticut. The only other host that has been reported is *Apantesis phalerata* Harr. (Krombein 1958). If the *Cratichneumon* found within a pupa on July 13 was deposited as an egg the previous fall, it might emerge late enough in the summer to attack new pupae in September.

Disease killed approximately 14% of the pupae each of the 2 years. The fewest diseased pupae were found at

South Windsor where the oakworm infestation was also of longest duration.

As the percentage loss of pupae from *C. w-album* and disease remained relatively constant between 1959 and 1960, the increased survival of the overwintering pupae in the winter of 1959–60 was probably caused by the decreased parasitism by *W. datanae*. The loss of pupae attributed to *W. datanae* dropped from 42.5% in the spring of 1959 to 16.4% in the spring of 1960. At Lebanon in 1959 where there were few pupae in the study plot (table 1) the percentage parasitized was lower than in any of the other plots, whereas the following year when there were comparatively more pupae there than elsewhere, it was the only study plot in which the per cent of moth emergence did not increase. Based on egg deposition on oakworm larvae as mentioned above and the comparative numbers of broken pupae in the spring, it would appear that *W. datanae* uaually attacks an increasingly larger percentage of the oakworm as the infestation proceeds but is less effective when the host population is low.

South Windsor was sprayed in the summer of 1959 with DDT to control the oakworm. Although the spray was poorly timed and was inadequate to prevent damage by the larvae, the number of pupae was greatly reduced that fall (table 1). From observations of the larval population it may be questioned if much of this was caused by the DDT spray. The following spring all of the pupae collected at this point emerged as adults, but as the oakworm population in the study plots was reduced to three pupae no possible conclusions could be drawn of the effects of DDT on pupal parasites.

It is difficult to assess the damage done by the orange-striped oakworm. Certainly in the scrubby low quality oak forest that covers much of Connecticut it is not economically feasible to treat large tracts of woodland. Damage to the larger forest trees is slight except in cases of severe outbreaks. Defoliation first occurs on border trees and usually on branches close to the ground. Thus feeding first occurs on young trees which would not likely be a future source of timber because they are overtopped by more mature trees.

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Longevity of the Boll Weevil under Laboratory Conditions[1]

EDWARD N. LAMBREMONT and N. W. EARLE, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

ABSTRACT

Two cultures (F_9 and F_{15}) of adult boll weevils (*Anthonomus grandis* Boheman) were caged in a temperature- and humidity-controlled room in trays containing cotton squares placed in moistened sand under a low-pressure mercury-vapor ultraviolet light. Another culture (F_1) was caged on squares on drier sand under a high-pressure mercury-vapor ultraviolet light. All weevils were fed fresh debracted squares changed daily. Survival data plotted for the three cultures approached the typical sigmoid or semirectangular curve characteristic of an animal population having a senescence. Longevity was variable, and depended upon sex, culture, and holding conditions. Greatest longevity was demonstrated by male weevils from Mexico with a mean of 121.5 days and a maximum of 199 days.

Several reports have appeared during the past 40 years regarding longevity in the adult boll weevil (*Anthonomus grandis* Boheman). These reports were concerned with survival in the uncontrolled environment of the field or the insectary (Howe 1916, Fenton & Dunnam 1929), or of the longevity of weevils following diapause (Newell & Dougherty 1909, Gaines 1959). We were prompted for several reasons to prepare a preliminary evaluation of boll weevil survival in the controlled laboratory environment without the interruption of a diapause period. This information is not available, and would be useful for evaluating research in various laboratories. Virtually all of the published work was concerned with field-collected as opposed to laboratory-reared weevils, and no information has been available about the survival of boll weevils reared on an artificial diet in the laboratory. The species is not among those listed by Spector (1956) in the table of insect longevities for either field- or laboratory-reared insects. It also seemed advisable to determine if boll weevil survival curves would exhibit the characteristic shape of a mortality curve for an animal population showing senescence.

MATERIALS AND METHODS.—All weevils were reared from egg to adult on an artificial diet in a temperature-controlled room at 27° C. and 40% relative humidity. The diet contained 10 grams of an acetone powder of cotton squares and 2 grams of soybean protein plus the standard larval-diet components described by Earle et al. (1959). All weevils for comparison within any particular culture were obtained from the same parental oviposition group, were reared together, and otherwise handled in an identi-

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[2] In cooperation with the Louisiana Agricultural Experiment Station. W. P. Rickey and J. A. Lambremont assisted with this research.

cal manner. The adults were held in different temperature, humidity, and illumination conditions that will be described where appropriate. All adults were fed fresh debracted cotton squares changed daily at the rate of one square per two weevils. The squares were placed stem-down in petri dishes of moistened sand, and the dishes were placed in cages made from screen-topped gallon ice-cream cartons. Not more than 100 adults (approximately 50 of each sex) were kept in a cage. Daily survival records were maintained.

Two cultures that had been maintained in the laboratory for different lengths of time were chosen to obtain most of the data for this study. One culture consisted of weevils from Mexico reared for nine successive generations, whereas the other was from Louisiana reared for 15 successive generations. Both cultures were held in the temperature-controlled room as described and were illuminated with a low-pressure mercury-vapor ultraviolet fluorescent lamp placed 12 inches above the sand trays in the cages. The temperature at the surface of the sand in cages so illuminated was measured with a Sargent[3] thermistor and averaged 26.4° C. The sand retained most of its moisture for 24 hours, but was moistened again each day when the squares were changed. The slight differential in temperature below that of the room probably resulted from cooling caused by evaporation. The relative humidity of the air in these cages averaged 45%.

RESULTS.—The minimum, maximum, and mean longevities for these two cultures are presented in table 1. Data are given for males and females held together and for females held without males. Males consistently had a longer lifespan than females. The slightly longer life of the Mexico females held without males as compared with those with males was not significant. These results compare well with those reported in the literature. One of the longest lifespans for this species for field-collected weevils (held in tumblers in the insectary) was 64 days for males and 57 for females (Fenton & Dunnam 1929).

An additional group of Louisiana weevils reared on larval diet in the laboratory for one generation was held under slightly less-favorable conditions. These weevils were placed in cages 24 inches beneath and slightly to the side of a high-pressure mercury-vapor ultraviolet lamp. The temperature at the surface of the sand averaged 29.7° C., and the sand dried out completely each 24 hours. The total lifespan under these conditions was less than with those weevils held at 26.4° C. with higher sand moisture, although the relative humidity of the air was found to be approximately the same as in the cages under fluorescent light. Longevity data for this culture are also presented in table 1.

Table 1.—Mean, maximum, and minimum adult longevities (days) of three boll weevil cultures reared on artificial larval diet in the laboratory.

CULTURE	NUMBER OF		LONGEVITY (DAYS)		
	Weevils	Weevil Days	Mean	Minimum	Maximum
Mexico					
F_9 Males	91	11,053	121.5	3	199
Females	79	6,073	76.9	1	168
Females held without males	50	4,190	83.8	4	170
Louisiana					
F_1 Males	38	2,636	69.4[a]	4	89
Females	29	1,590	54.8	4	82
F_{15} Males	63	5,607	89.0	1	180
Females	56	3,841	68.6	1	153

[a] Terminated on the 89th day with eight males still living, thus the maximum and mean longevities are somewhat longer than shown.

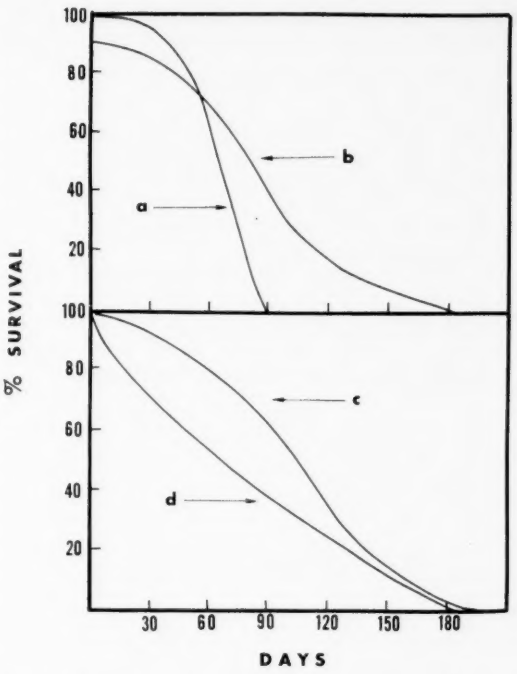

FIG. 1.—Survival curves of boll weevil cultures compared with the theoretical curve of an animal population not possessing a senescence. Curve *a*, Louisiana culture one generation removed from the field; *b*, Louisiana culture 15 generations; *c*, Mexico culture 9 generations; *d*, hypothetical curve of a nonsenescing animal population (Comfort 1954).

Survival Curves.—Mortality data were pooled for both sexes of the one Mexico and two Louisiana cultures, and survival curves were plotted. The survival curves for the three cultures (fig. 1*a*, 1*b*, and 1*c*) approach the typical sigmoid or semirectangular-shaped curve characteristic of an animal population having a senescence (Comfort 1954). This is described as a slow death rate of the younger individuals followed by an accelerated mortality as age progresses. The curve for the Louisiana weevils only one generation removed from the field (fig. 1*a*) possessed the most rectangular shape of the three, whereas that for weevils from Mexico approached the logarithmic shape (fig. 1*c*). For comparative purposes a curve has been included (fig. 1*d*) that is the type obtained when mortality data of an animal population not possessing a senescence are plotted.

DISCUSSION.—It is evident from this and other studies that boll weevil longevity is quite variable depending upon sex, culture, and holding conditions. In general, the

[3] Mention of a proprietary product does not imply endorsement by the U. S. Department of Agriculture.

lifespan obtained for this study exceeds that for adults held in the insectary or in field cages. It is expected that the lifespan could be prolonged even further by rearing and holding at lower temperatures.

Care must be taken in choosing insects for comparative studies of different aged weevils. For instance, some researchers have used 10-day-, 20-day-, and 40-day-old adults as young, middle-aged, and senescent weevils, respectively. It is evident that even the 40-day-old weevils are relatively young when compared with the mean lifespan of the typical individual.

Eggs obtained from the Mexico females when they were 120 to 130 days old were found to be as viable as those from younger females (5 to 30 days old). This evidence indicates that boll weevils can be reared on an artificial diet continuously in the laboratory without impairment to longevity or fecundity.

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Field Experiments for Control of Thrips, Cotton Fleahoppers, and Overwintered Boll Weevils in 1960[1]

J. W. DAVIS, C. R. PARENCIA, JR., and C. B. COWAN, JR., *Entomology Research Division, Agric. Res. Serv., U.S.D.A.,*[2] *Waco, Texas*

ABSTRACT

Sevin, Geigy 30494, toxaphene, Strobane, and toxaphene plus DDT were equally effective against thrips (*Frankliniella* spp.). Toxaphene plus DDT gave significantly better control of the cotton fleahopper (*Psallus seriatus* (Reuter)) than toxaphene alone or Strobane. Bayer 29493 gave good control of this pest, Shell SD-4402 poor control, and Geigy 30494 was ineffective. Good kills of overwintered boll weevils (*Anthonomus grandis* Boheman) were obtained with Shell Compounds SD-5539 and SD-5533, Geigy 30494, Methyl Trithion, and Bayer 29493. Bayer 30911 was ineffective. Am. Cyanamid Anti-feeding Compound 24055 was ineffective against thrips, the cotton fleahopper, and overwintered boll weevils.

The chemical definitions of the insecticides with proprietary names mentioned above are: Strobane® (a mixture of chlorinated terpenes with about 66% of chlorine); Sevin® (1-naphthyl *N*-methylcarbamate); Geigy 30494 (*O,O*-dimethyl *S*-(2,5-dichlorophenylthio) methyl phosphorodithioate); Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate); Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan); Shell SD-5539 (dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate); Shell SD-5533 (dimethyl 1-(*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate); Methyl Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate); Bayer 30911 (*O*-(2,4-dichlorophenyl) *O*-methyl methylphosphonothioate); Am. Cyanamid Anti-feeding Compound 24055 (4′-(dimethyltriazeno) acetanilide).

In 1960 field-cage experiments were conducted in cotton fields in central Texas to evaluate the effectiveness of several new insecticides against overwintered boll weevils (*Anthonomus grandis* Boheman). Field-plot experiments were also conducted to evaluate new insecticides against thrips (*Frankliniella* spp.), the cotton fleahopper (*Psallus seriatus* (Reuter)), the boll weevil, the bollworm (*Heliothis zea* (Boddie)), and the desert spider mite (*Tetranychus desertorum* Banks).

FIELD-CAGE EXPERIMENTS AGAINST OVERWINTERED BOLL WEEVILS.—Four experiments were conducted in single plots 16 rows wide and 1 to 2 acres in size, located in a small early-planted field. Most fields in the area had to be replanted but this one did not, and as a result large numbers of boll weevils were present.

Procedures used in conducting the experiments were the same as those reported by Parencia et al. (1960). Twenty boll weevils were caged per treatment in each experiment.

The results of these experiments are given in table 1. In experiment 1 good kills were obtained with Shell SD-5533 (dimethyl 1-(*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate), Shell SD-5539 (dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate), and toxaphene plus DDT. Strobane® (a mixture of chlorinated terpenes with about 66% of chlorine) plus DDT and Geigy 30494 (*O,O*-dimethyl *S*-(2,5-dichlorophenylthio) methyl phosphorodithioate) were less effective. Kill with Bayer 30911 (*O*-(2,4-dichlorophenyl) *O*-methyl methylphosphonothioate) was poor.

The insecticides used in experiment 1 were retested in experiment 2; however, dosages of Geigy 30494 and Bayer 30911 were increased. All of the insecticides except Bayer 30911 gave 100% kill.

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[2] In cooperation with the Texas Agricultural Experiment Station.

In experiment 3 good kills were obtained with all insecticides, although Bayer 29493 (*O,O*-dimethyl *O*-(4 methylthio-*m*-tolyl) phosphorothioate) and Sevin®(1-naphthyl *N*-methylcarbamate) plus Ortho[3] spray sticker were somewhat less effective than the other insecticides.

In experiment 4 the same insecticides were used as in experiment 3 and a lower dosage of Methyl Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate) was included. The lower dosage of Methyl Trithion was less effective than the higher dosage in both experiments. The difference in kill obtained with Sevin with the sticker compared with Sevin alone was greater than in the previous experiment.

FIELD-PLOT EXPERIMENTS AGAINST SEVERAL COTTON PESTS.—Six field-plot experiments were conducted. In experiment 1 the plots were 0.2 acre in size arranged in a randomized block with four replicates. Untreated checks were not included in the experimental design and were located adjacent to the treated plots.

Experiments 2–6 consisted of single plots 16 to 32 rows wide ranging from 1 to 3 acres in size. Methods of applying insecticides early in the season were the same as those reported by Davis et al. (1957). Applications were made at about 7-day intervals. Late-season applications were made in accordance with those reported by Cowan et al. (1956) at approximately 4- to 5-day intervals.

Procedures used in recording data were the same as those reported by Ewing & Parencia (1943). Data on control of early- and late-season insects in the same experiments are given in tables 2 and 3, respectively.

In experiment 1, the first four early-season applications were made for thrips control. All insecticides were effective. The last three early-season applications were made at a higher dosage for the control of all early-season insects. Sevin with or without Ortho spray sticker and toxaphene plus DDT gave significantly better control of the cotton fleahopper than toxaphene or Strobane; Geigy 30494 was no more effective than the latter materials. Results obtained in 1958 and 1959 also showed that

Table 1.—Effectiveness of several insecticides against overwintered boll weevils in field-cage experiments.

Insecticide (lb./gal.)	Toxicant per Acre (lb.)	Kill at Indicated Days After Treatment (%) 1	5
Experiment 1 (Treated on May 27)			
Toxaphene, 4+DDT, 2	1.5+0.75	5.3	89.5
Strobane, 4+DDT,2	1.5+ .75	0	77.8
Geigy 30494, 2	0.25	20.0	80.0
Shell SD-5539, 2	.25	57.1	95.0
Shell SD-5533, 2	.25	65.0	100.0
Bayer 30911, 4	.5	0	26.1
Untreated	—	—	—
Experiment 2 (Treated on June 2)			
Toxaphene, 4+DDT, 2	1.5+0.75	38.9	100.0
Strobane, 4+DDT, 2	1.5+ .75	50.0	100.0
Geigy 30494, 2	0.5	100.0	—
Shell SD-5539, 2	.25	90.0	100.0
Shell SD-5533, 2	.25	100.0	—
Bayer 30911, 4	1.0	11.1	28.5
Untreated	—	—	—
Experiment 3 (Treated on June 2)			
Methyl parathion, 2	0.25	94.4	100.0
Methyl Trithion, 4	.5	85.0	100.0
Sevin,[a]+sticker[b]	1.25	50.0	93.0
Sevin[a]	1.25	94.4	100.0
Bayer 29493, 4	0.5	60.0	95.5
Untreated	—	—	11.1
Experiment 4 (Treated on June 9)			
Methyl parathion, 2	0.25	95.0	95.0
Methyl Trithion, 4	.5	89.5	89.5
	.25	65.0	80.0
Sevin,[a]+sticker[b]	1.25	55.6	72.2
Sevin[a]	1.25	89.5	100.0
Bayer 29493, 4	0.5	100.0	—
Untreated	—	—	—

[a] 85%, wettable powder.
[b] Ortho spray sticker at 4 fluid ounces per acre.

Table 2.—Effectiveness of several insecticidal sprays against thrips, the cotton fleahopper, and overwintered boll weevils in field-plot experiments.

Insecticide (lb./gal.)	Toxicant per Acre (lb.)	Number of: Thrips per Plant	Fleahoppers per 100 Terminals	Boll Weevil Punctured Squares (%)
Experiment 1 (7 applications between May 4 and June 16[a])				
Sevin[b]	0.82	0.7	14.7	1.7
Sevin[b]+sticker[c]	.82	1.3	14.4	2.6
Geigy 30494, 2	.36	0.7	23.2	3.1
Strobane, 6	1.82	.9	28.3	2.9
Toxaphene, 6	1.82	.5	28.9	3.5
Toxaphene, 4+DDT,2	1.2+0.6	.8	16.8	2.9
L.S.D. at 5% level	—	—	7.3	—
Untreated (Outside Check)	—	9.4	29.5	6.0
Experiment 2 (7 applications between May 4 and June 16[a])				
Sevin[b]	0.82	0.8	9.6	2.3
Sevin[b]+sticker[c]	.82	.3	10.8	2.8
Toxaphene, 4+DDT, 2	1.2+0.6	.3	12.4	3.7
Untreated	—	9.4	29.5	6.0
Experiment 3 (5 applications Between May 16 and June 13)				
Am. Cyanamid 24055[d]	3.0	1.1	28.6	40.5
	1.5	1.6	29.2	42.9
Am. Cyanamid 24055[d]+ toxaphene, 4+DDT, 2	1.5+0.5+0.25	1.8	12.5	33.4
Methyl parathion, 2	0.4	1.6	7.8	24.9
Toxaphene, 4+DDT, 2	1.8+0.9	0.1	3.6	14.3
Untreated	—	1.8	24.9	45.1
Experiment 4 (3 applications between May 28 and June 10)				
Toxaphene, 4+DDT, 2	1.4+0.7	—	3.2	19.6
Strobane, 4+DDT, 2	1.4+ .7	—	7.1	13.7
Geigy 30494, 2	0.4	—	30.3	9.0
Shell SD-4402, 1.25	.4	—	12.0	10.6
Sevin[b]	1.1	—	11.0	11.9
Untreated	—	—	31.9	26.6
Experiment 5 (3 applications between May 27 and June 9)				
Toxaphene, 4+DDT, 2	1.7+0.85	—	1.7	9.0
Strobane, 4+DDT, 2	1.7+ .85	—	2.5	8.3
Geigy 30494, 2	0.4	—	9.7	8.0
Shell SD-5539, 2	.3	—	11.0	11.0
Shell SD-5533, 2	.3	—	8.1	20.5
Bayer 30911, 4	.8	—	5.5	48.1
Untreated	—	—	5.0	52.7
Experiment 6 (1 application on June 17)				
Bayer 29493, 4	0.25	—	12.5	1.9
Shell SD-4402, 1.25	.25	—	33.1	2.6
Methyl Trithion, 4	.25	—	42.7	7.2
Methyl parathion, 2	.25	—	30.7	2.1
Sevin[b]	.75	—	20.1	1.1
Toxaphene, 4+DDT, 2+ Methyl parathion, 2	1.0+0.5+0.125	—	15.1	1.0
Toxaphene, 4+DDT, 2	1.0+0.5	—	26.9	1.8
Untreated	—	—	60.3	5.2

[a] Dosages are averages of thrips and cotton fleahopper treatments.
[b] 85%, wettable powder.
[c] Ortho spray sticker at 4 fluid ounces per acre.
[d] 25%, wettable powder.

[3] Mention of proprietary products does not necessarily imply their endorsement by the U. S. Department of Agriculture.

Table 3.—Effectiveness of several insecticidal sprays against three late-season cotton insects in field-plot experiments.

Insecticide (lb./gal.)	Toxicant per Acre (lb.)	Desert Spider Mites per Sq. Inch (no.)	Boll Weevil Punctured Squares (%)	Bollworm Injured (%)		Seed Cotton per Acre (lb.)	
				Squares	Bolls	Total	Gain Over Check
Experiment 1 (9 spray applications between July 7 and August 16)							
Sevin[a]	1.4	3.7	21.5	2.2	3.8	1,399	942
Sevin[a]+sticker[b]	1.4	1.7	26.5	2.6	4.9	1,112	655
Geigy 30494, 2+DDT, 2	0.5+1.3	0	31.8	3.9	6.8	1,026	569
Strobane, 4+DDT, 2	2.5+1.25	0.4	33.6	1.9	3.4	1,208	751
Toxaphene, 4+DDT, 2	2.5+1.25	.5	31.2	2.6	3.8	1,115	658
Toxaphene, 4+DDT, 2	2.5+1.25	1.2	36.2	2.1	4.8	1,271	814
L.S.D. at 5% level	—	1.3	—	0.7	1.7	—	—
Untreated (Outside Check)	—	0.02	44.4	6.4	9.8	457	—
Experiment 2 (10 spray applications between July 7 and August 16)							
Sevin[a]	1.4	5.2	9.8	2.4	4.5	1,357	900
Sevin[a]+sticker[c]	1.4	3.3	11.2	2.1	3.6	1,209	752
Toxaphene, 4+DDT, 2	2.4+1.2	1.7	29.0	2.3	4.1	1,389	932
Untreated	—	0.02	33.9	6.4	9.8	457	—
Experiment 3 (15 applications between June 21 and August 19)							
Toxaphene, 4+DDT, 2+ methyl parathion, 2	2.0+1.0+0.25	—	36.6	3.1	2.7	1,617	1,432
Toxaphene, 4+DDT, 2+ methyl parathion, 2	2.0+1.0+ .25	—	44.9	3.4	3.5	1,566	1,381
Toxaphene, 4+DDT, 2	2.6+1.3	—	52.3	3.3	3.0	1,695	1,510
Methyl parathion, 2+DDT, 2	0.5+1.0	—	45.7	5.5	5.1	1,616	1,431
Toxaphene, 4+DDT, 2	2.5+1.25	—	35.2	2.8	2.7	1,652	1,469
Untreated	—	—	69.6	14.5	19.1	185	—
Experiment 4 (7 applications between June 30 and July 27)							
Toxaphene, 4+DDT, 2	2.4+1.2	1.2	39.0	1.1	1.4	1,638	1,106
Strobane, 4+DDT, 2	2.4+1.2	1.3	24.9	2.0	1.6	1,588	1,056
Geigy 30494, 2+DDT, 2	0.5+1.0	0	17.8	1.9	2.6	1,450	918
Shell SD-4402, 1.25+methyl parathion, 2	.5+0.25	1.8	37.2	1.4	1.0	1,564	1,032
Sevin[a]	1.25	5.5	17.0	1.2	1.0	1,756	1,224
Untreated	—	0.8	56.6	4.5	15.5	532	—
Experiment 5 (7 applications between June 22 and July 26)							
Toxaphene, 4+DDT, 2	2.0+1.0	6.5	22.1	0.8	0.6	1,596	1,096
Strobane, 4+DDT, 2	2.0+1.0	5.1	16.6	.5	.4	1,484	984
Geigy 30494, 2+DDT, 2	0.5+1.0	0	12.0	1.3	1.8	1,596	1,096
Shell SD-5539, 2+DDT, 2	.5+1.0	0	11.0	1.2	0.4	1,484	984
Shell SD-5533, 2+DDT, 2	.5+1.0	0	27.0	0.9	1.2	1,407	907
Toxaphene, 4+DDT, 2+ methyl parathion, 2	2.0+1.0+0.25	0	49.6	.9	1.8	1,253	753
Untreated	—	0	76.0	.9	1.9	500	—
Experiment 6 (10 applications between July 6 and August 19)							
Bayer 29493, 4+DDT, 2	0.5+1.5	—	12.1	6.6	4.7	1,783	758
Shell SD-4402, 1.25+ methyl parathion, 2	.5+0.25	—	20.0	3.0	2.0	1,871	846
Methyl Trithion, 1+DDT, 2	.5+1.0	—	10.8	4.1	3.5	1,927	902
Methyl parathion, 2+DDT, 2	.4+1.0	—	13.6	2.6	2.9	1,832	807
Sevin[a]	1.25	—	7.1	2.8	2.8	2,197	1,172
Toxaphene, 4+DDT, 2+ methyl parathion, 2	2.0+1.0+0.25	—	10.4	2.1	2.2	2,011	986
Toxaphene, 4+DDT, 2	2.5+1.25	—	18.8	2.4	2.8	2,029	1,004
Untreated	—	—	34.6	8.3	7.2	1,025	—

[a] 85%, wettable powder.
[b] 6 fluid ounces of tung oil.
[c] Ortho spray sticker at 4 ounces per acre.

DDT in mixtures with toxaphene or Strobane or some of the other clorinated hydrocarbons gave better control than toxaphene or Strobane alone (Parencia et al. 1960, Parencia & Cowan 1960).

For late-season control beginning on July 7 the same treatments were used at increased dosages except that DDT was added to Geigy 30494, Strobane, and toxaphene. Six fluid ounces of tung oil plus 1% of Triton X-100[3] were used with the Sevin treatment instead of the Ortho spray sticker. No significant difference in boll

weevil control was shown between insecticides. All of the other formulations were more effective against the bollworm than Geigy 30494 plus DDT. Infestations of the desert spider mite developed in mid-July in all of the treated plots except those treated with Geigy 30494 plus DDT. Significantly heavier infestations were found in the plots treated with Sevin without a sticker than in those with the other insecticides. In the July 16 application demeton was added to all treatments except Geigy 30494 plus DDT for desert spider mite control. No significant difference in yield was shown between treatments; however, yields were lower in plots treated with insecticides that had given poor cotton fleahopper contol.

In experiment 2 conducted in the same field as experiment 1 no appreciable difference in thrips or cotton fleahopper control was shown between insecticides. Overwintered boll weevil populations were very light.

The same treatments at increased dosages were used for late-season insect control. Both Sevin treatments were more effective against the boll weevil than toxaphene plus DDT. No difference was shown in bollworm control. Desert spider mite infestations developed in all treated plots in mid-July. They were heavier in plots treated with Sevin than in those with toxaphene plus DDT. As in experiment 1 demeton was added to each insecticide in the July 16 application for desert spider mite control. Good increases in yield over the untreated check were obtained with all insecticides.

In experiment 3, Am. Cyanamid Anti-feeding Compound 24055 (4′-(dimethyltriazeno) acetanilide) was ineffective against thrips. The cotton fleahopper infestation was light when treatments were started but increased considerably thereafter. Am. Cyanamid 24055 was ineffective against this pest. A mixture of this material with toxaphene and DDT gave some control but not as much as toxaphene plus DDT or methyl parathion. Differences in control were reflected in fruiting records. The average of five records on the number of fruiting forms per foot of row between June 1 and 20 in the different treatments was as follows: Untreated check, 3.3; Am. Cyanamid 24055 at 3 and 1.5 pounds per acre, 2.9 and 3.4 respectively; Am. Cyanamid 24055 plus toxaphene plus DDT, 5.0; methyl parathion, 6.0; and toxaphene plus DDT, 7.0. Am. Cyanamid 24055 was ineffective against overwintered boll weevils. Good control was obtained with methyl parathion and toxaphene plus DDT.

For late-season insect control plots treated with Am. Cyanamid 24055 were treated with toxaphene plus DDT plus methyl parathion. The plot receiving the early-season combination of Am. Cyanamid 24055 plus toxaphene plus DDT was treated later with toxaphene plus DDT; DDT was added to methyl parathion in the plot treated with methyl parathion; and toxaphene plus DDT continued to be used on the plot originally receiving this treatment. Late-season treatment was started on June 21 with 15 applications made through August 19; however, the plot treated with toxaphene plus DDT throughout the season did not receive the last two applications. The late-season boll weevil infestation was owing to population buildup in plots that had received early-season treatments of Am. Cyanamid 24055. The most effective seasonal control was obtained with toxaphene plus DDT used throughout the season and with toxaphene plus DDT plus methyl parathion for late-season control. Methyl parathion plus DDT was slightly more effective than toxaphene plus DDT used only for late-season control. Three of the applications between July 25 and August 2 were made at 3-day intervals. The average application interval was approximately 4 days. Each of the other insecticides gave better control of a heavy bollworm infestation than methyl parathion plus DDT.

Maturity of plants receiving early-season applications of methyl parathion and late-season applications of this material plus DDT was delayed 7 to 10 days over that of plants receiving toxaphene plus DDT throughout the season. On August 24, 67% of the bolls were open in the plot receiving the latter treatment whereas only 48% were open in the plot receiving methyl parathion. The first yield record made on September 1 showed that almost 300 more pounds of seed cotton per acre were picked from the toxaphene-plus-DDT plot than from the one treated with methyl parathion plus DDT. Similar results were obtained in 1959 (Parencia et al. 1960). Little difference was shown in final yields between insecticides.

In experiment 4 little difference occurred in overwintered boll weevil control between insecticides. Each insecticide except Geigy 30494 gave good control of the cotton fleahopper; however toxaphene plus DDT gave the most effective control.

For late-season control the plots received the same insecticides at increased dosages except that DDT was added to Geigy 30494 for bollworm control and methyl parathion was added to Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4, 7-methanophthalan) for boll weevil control. Sevin and Geigy 30494 plus DDT gave the most effective late-season boll weevil control. The infestation in the Shell SD-4402 plot rose rapidly during the treatment period; it was necessary to add methyl parathion in the last three applications to avoid boll weevil damage to bolls. Fair-to-good control was obtained with Strobane or toxaphene plus DDT. All insecticides gave good bollworm control. A heavy spider mite infestation developed in the Sevin plot and medium infestations developed in the plots treated with toxaphene plus DDT, Strobane plus DDT, and Shell SD-4402. Demeton was added to these treatments in the application made on July 14 for desert spider mite control. Good increases in yield were obtained with all treatments. The somewhat lower yield obtained with Geigy 30494 plus DDT was because of the poor cotton fleahopper control obtained with this material alone earlier in the season.

In experiment 5, Bayer 30911 was ineffective against overwintered boll weevils. Cotton fleahopper infestations were too light to evaluate the effectiveness of the treatments against this pest.

The same treatments were used for late-season insect control except that toxaphene plus DDT plus methyl parathion was used instead of Bayer 30911, and DDT was added to Geigy 30494, Shell SD-5539, and Shell SD-5533 for bollworm control. All of the insecticides gave good boll weevil control although the toxaphene plus DDT plus methyl parathion treatment had the highest average infestation (49.6%). However, an infestation of 48.1% existed when the initial application of this material was made. These data emphasize the importance of reducing overwintered weevil populations in maintaining

late-season control of this pest. All of the insecticides gave good bollworm control. Infestations of the desert spider mite and of the cotton aphid (*Aphis gossypii* Glover) developed only in the plots treated with toxaphene plus DDT and Strobane plus DDT. Demeton was added to these treatments in the July 11 application to control these pests. The greatest gains in yield were obtained with toxaphene plus DDT and Geigy 30494 plus DDT. The lowest gain was obtained with toxaphene plus DDT plus methyl parathion and reflected the failure of Bayer 30911 to control overwintered boll weevils. Dry weather prevailed during the late-season period and because of a heavy weevil infestation plants were unable to replace the fruit lost earlier in the season to overwintered weevils.

In experiment 6, 6 days following one application of the insecticides for cotton fleahopper control, Bayer 29493 and toxaphene plus DDT plus methyl parathion were giving the best control. Poorest control was obtained with Shell SD-4402 and Methyl Trithion. Because of rain the second application could not be made. The infestation remained below injurious levels in all but the Shell SD-4402, Methyl Trithion, and methyl parathion treatments between the date of treatment and July 6, when late-season treatment for bollworm control began.

The same materials were used for late-season insect control except that DDT was added to Methyl Trithion and methyl parathion. DDT was added to Bayer 29493 beginning with the third application when it became evident that this material alone would not control the bollworm. Methyl parathion was included with Shell SD-4402 beginning with the sixth application for improved boll weevil control. The boll weevil infestation in this experiment did not become heavy until the end of July. Sevin, toxaphene plus DDT plus methyl parathion, Bayer 29493 plus DDT, Methyl Trithion plus DDT, and methyl parathion plus DDT gave excellent control. The bollworm infestation was severe during the early treatment period. Each insecticide gave good bollworm control except Bayer 29493. Blanket treatments by airplane with toxaphene plus DDT plus methyl parathion were made on August 25, 30 and September 5 for boll protection when wet conditions of the field and lodged plants prevented further operation of ground equipment. The smallest increase in yield was obtained with Bayer 29493 plus DDT, which reflected the lack of bollworm control in the early applications when this material was used without DDT.

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Effect of Curly Top and Cucumber Mosaic Viruses on Cantaloups[1]

ORIN A. HILLS,[2] DONALD L. COUDRIET,[2] ROSS W. BRUBAKER,[2] and PAUL D. KEENER[3]

ABSTRACT

Cantaloup plants were inoculated in experimental plots with cucumber mosaic virus (CMV) and curly top virus (CTV) separately and in combination. CMV is transmitted predominantly by the green peach aphid (*Myzus persicae* (Sulzer)) and CTV only by the beet leafhopper (*Circulifer tenellus* (Baker)). Inoculations were made when plants were in the six-leaf stage of development. Plants inoculated with CMV, either alone or in combination with CTV, were smaller and produced smaller melons. Size of plants or melons was not affected by CTV alone. Yield of marketable melons was reduced 32% over the uninoculated checks for CTV alone, 40% for CMV, and 68% in plots inoculated with both viruses.

Several insect-transmitted virus diseases are known to affect cantaloups in southern Arizona and southern California. Curly top, transmitted by the beet leafhopper (*Circulifer tenellus* (Baker)), sometimes limits cantaloup production in these areas. Hills & Taylor (1949) showed the effect of the disease under controlled conditions on young cantaloup plants. Later the same authors (1954) showed that younger plants were more severely affected than older plants. Flock et al. (1960) reported crop failures from curly top in the Imperial Valley of California in 1958 and further demonstrated the effect of certain strains of this virus on the crop in 1959.

Several mosaic-type viruses affecting cantaloups and other melons have been recognized for some time, although there has been considerable confusion in their identity. Grogan et al. (1959) states that the two most prevalent mosaic viruses infecting cucurbits in southern California from 1956 to 1958 were watermelon mosaic and cucumber mosaic. These same two viruses have been isolated from cantaloup plants in the Yuma and Salt River Valleys of Arizona and are also recognized as the most prevalent mosaic viruses affecting cantaloups in this State. Both viruses are transmitted by aphids, of which the green peach aphid (*Myzus persicae* (Sulzer)) is the most important.

METHODS.—In order to determine more specifically the

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[2] Entomology Research Division, Agricultural Research Service, U.S.D.A.

[3] University of Arizona, College of Agriculture, Department of Plant Pathology. (Ariz. Agric. Expt. Sta. Technical paper No. 641.)

FIG.

effects on cantaloups of curly top virus (CTV) and cucumber mosaic virus (CMV) alone and in combination, small-plot tests were made at Mesa, Arizona on cantaloups of the Powdery Mildew Resistant No. 45 variety. Four inoculation treatments and an untreated check were replicated in six randomized blocks. Each plot contained 10 plants and inoculations were made at the six-leaf stage of development by caging infective beet leafhoppers on the plants. Earlier inoculations were avoided to prevent complete destruction of the plants by curly top. Cages were made from half-gallon ice-cream cartons cut in two sections. One end was covered with a fine-mesh scrim, and a series of 1-inch holes cut toward the bottom were also covered with scrim. These made cylindrical cages $4\frac{1}{2}$ by $4\frac{1}{2}$ inches. After the cages had been placed over the plants, 5 to 10 infective leafhoppers from colonies on curly top beets were placed in each cage. The inoculum source was propagated in the greenhouse from a few sugar beets showing curly top symptoms in the field. The strain of CTV was, therefore, unknown. After 48 hours the leafhoppers were killed by cyanide fumigation and the cages removed.

Cucumber mosaic virus inoculations were made mechanically by grinding runner tips of cantaloup plants and leaves of sugar beets, both showing CMV symptoms, in a food chopper and straining the juice through muslin. The undiluted juice, without buffer, was then rubbed into the leaves of the plants in the field plots with carborundum; after 10 minutes the leaves were washed with distilled water.

Criteria used to measure the effect of these viruses on the plants were: (1) Plant growth as determined by runner measurements; (2) yield of melons; (3) numbers of culls as compared with marketable melons; (4) size of melons; and (5) quality as indicated by total soluble solids in the juice. As the melons were picked they were divided into culls and marketable melons. The marketable ones were then classified as 45's, 36's, and 27's or larger. These sizes represent the number of melons packed in a commercial crate. The percentage of total soluble solids was determined for marketable melons only. This procedure was accomplished in the laboratory by plugging all melons from each plot on each harvest date. A $\frac{7}{8}$-inch cork borer was used, and plugs were taken midway between the blossom and stem end of the melon. The rind was removed and the aggregate of all plugs from each plot on each harvest date was blended in a kitchen blender and the juice filtered off. The percentage of total soluble solids was then determined with a refractometer.

Disease readings were attempted in all plots, but curly top symptoms in cantaloups are so unreliable that these data were discarded. CMV symptoms on the other hand were definite, and the data were recorded.

RESULTS.—Ten days after inoculations with CMV, plants had runners up to 12 inches long and disease symptoms were beginning to show. Readings taken 13 and 28

FIG. 1.—One replicate of plots experimentally inoculated with CMV and CTV. Plants were inoculated at the six-leaf stage as follows: P, uninoculated check; O, CMV+CTV; M, CTV; N, CMV. Photo June 10, 28 days after inoculation.

Table 1.—Cucumber mosaic symptoms and runner length of plants inoculated at 6-leaf stage with CMV and CTV (10 plants per plot.)[a]

Inoculation Material	Number of Plants with CMV Symptoms Days After Inoculation		Runner Length 19 Days After Inoculation (inches)
	13	28	
CMV	9.2 a	9.8 a	13 a
CMV and CTV	9.0 a b	9.5 a	15 a
CTV	0.0 b	1.3 b	24 b
None	0.0 b	0.3 c	25 b

[a] Values not followed by the same letter are significantly different at the 5% level. Duncan's multiple range test (1955).

Table 2.—Growth and production of melons from plants inoculated at 6-leaf stage with CMV and CTV (10 plants per plot).[a]

Inoculation Material	No. of Marketable Melons per Plot	No. of Melons Per Crate	Total Soluble Solids (%)
CTV and CMV	7 a	38 a	9.26 a
CMV	13 b	37 a	9.21 a
CTV	15 b	33 b	9.82 a
None	22 c	32 b	9.63 a

[a] Values not followed by the same letter are significantly different at the 5% level.

days after inoculations are given in table 1. These data show that 98% of the plants had developed mosaic symptoms 28 days after inoculation with CMV compared with 3% of those in the uninoculated check plots. Plots inoculated with CTV alone showed 13% of the plants had developed mosaic symptoms at this time, which was significantly more than in the uninoculated checks.

Data from runner measurements are also given in table 1. Significant reductions in runner length over plants in the untreated check plots were shown 19 days after inoculation with CMV either alone or in combination with CTV. No reductions in runner length were shown for plants inoculated with CTV alone. These reductions in growth caused obvious differences in the appearance of the plots for the rest of the season. Figure 1 shows one replicate of these plots as they appeared on June 10.

Yield of marketable cantaloups, average size of the melons, and total soluble solids in the juice are given in table 2. These data show a 32% reduction over the uninoculated checks in yield of marketable melons from plants inoculated with CTV alone, 41% reduction from CMV alone, and 68% reduction from plants inoculated with both viruses. No significant differences between treatments in numbers of cull melons were indicated, but melons from plants inoculated with CMV alone, or in combination with CTV, were significantly smaller than melons from the CTV plots or those from uninoculated check plots. No significant differences were shown in total soluble solids of melons from any of the plots, but some tendency toward a reduction in soluble solids resulted from CMV inoculations either with or without CTV.

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Field Tests with Insecticides for Pink Bollworm Control in 1958[1]

R. L. McGarr, A. J. Chapman, and W. L. Lowry, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.,*[2] *Brownsville, Texas*

Abstract

In experiments conducted at Brownsville, Texas, in 1958, Sevin® (1-naphthyl *N*-methylcarbamate), a mixture of Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) plus DDT, and a BHC-DDT mixture were highly effective against the pink bollworm (*Pectinophora gossypiella* (Saunders). Bayer 29493 (*O,O*-dimethyl *O*-[4-methylthio-*m*-tolyl] phosphorothioate) and Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate) were effective against the pink bollworm but not effective against the bollworm (*Heliothis zea* (Boddie)). General Chemicals 3707 (1,3-bis (methoxycarbonyl)-1-propen-2-yl dimethyl phosphate) and Monsanto CP-7769 (hexaethyl (ethylthiomethylidyne)triphosphonate) were not effective against either of these insects. Barthrin showed some promise for control of the pink bollworm but not the bollworm.

Experiments were conducted to evaluate promising new insecticides and to compare several commonly used insecticides and mixtures for control of cotton insects in the lower Rio Grande Valley. The experiments were planned primarily for control of the pink bollworm (*Pectinophora gossypiella* (Saunders)). In addition, infestation records were made for the boll weevil (*Anthonomus grandis* Boheman), bollworm (*Heliothis zea* (Boddie)), and cotton fleahopper (*Psallus seriatus* (Reuter)).

Methods.—Four experiments were conducted, three on small plots and one on large plots. The small-plot experiments included two with dusts and one with emulsion sprays. Dust treatments were applied to randomized plots measuring 0.2 of an acre. The sprays were applied to plots measuring 0.1 of an acre. The treatments were replicated four times. The insecticides tested included Sevin® (1-naphthyl *N*-methylcarbamate), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-)4*H*)-ylmethyl)

[1] Accepted for publication April 17, 1961.

[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Insecticide applications, seasonal infestation averages, and yields for pink bollworm, boll weevil, and bollworm control tests in the lower Rio Grande Valley in 1958.

Treatment	Average Toxicant per Acre (lb.)	Pink Bollworm Infested (%) Blooms[a]	Pink Bollworm Infested (%) Bolls	Pink Bollworm Number of Mines per Boll	Boll Weevil Squares Punctured (%)	Bollworm Injured (%) Squares	Bollworm Injured (%) Bolls	Yield of Seed Cotton (lb./acre) Total	Yield of Seed Cotton (lb./acre) Gain
Small-plot tests									
Experiment 1, dusts (9 applications[b])									
Check		24.2	50.7	1.26	8.8	4.5	3.8	894	
Sevin, 5%	1.2	25.8	17.6	0.34	0.7	1.5	0.5	1,860	966
10%	2.3	22.9	7.7	.12	1.4	1.1	0	2,142	1,248
Guthion, 2.5%+DDT, 5%	0.6+1.2	21.7	11.8	.22	1.1	1.4	2.3	2,052	1,158
L.S.D. at 5% level			7.1					610	
Experiment 2, dusts (7 applications[c])									
Check		—	48.8	1.21	17.0	5.6	7.3	1,626	
Bayer 29493, 5%	1.3	—	17.5	0.32	11.6	3.9	5.8	2,285	659
Bayer 25141, 2%	0.4	—	15.8	.24	13.8	6.0	5.8	2,156	530
BHC, 3%+DDT, 15%	0.8+4.1	—	15.0	.22	10.9	1.3	2.8	2,758	1,132
L.S.D. at 5% level			18.5			2.3	1.2	773	
Experiment 3, sprays (7 applications[d])									
Check		5.4	59.0	1.59	8.4	4.7	6.8	1,228	—
Bayer 25141	0.5	5.4	19.7	0.42	5.3	4.8	4.9	2,374	1,146
Barthrin	2.0	5.4	34.9	.79	4.8	2.8	3.3	1,866	638
G.C. 3707	0.5	5.4	54.1	1.33	4.6	5.1	5.6	1,374	146
CP-7769	.5	5.4	52.6	1.27	4.0	4.6	5.3	1,516	288
Guthion+DDT	0.5+0.5	5.4	29.6	0.56	3.4	2.1	2.5	2,306	1,078
L.S.D. at 5% level			7.7			3.9	2.8	574	
Large-plot tests									
Experiment 4, dusts (8 applications[e])									
Check		46.1	75.7	3.09	1.1	2.1	—	330	
Sevin, 10%	2.3	45.2	4.7	0.08	0.2	0.3	—	2,103	1,773
Guthion, 2.5%+DDT, 5%	0.6+1.1	46.2	3.7	.05	.1	.5	—	2,175	1,845
L.S.D. at 5% level			10.3					650	

[a] Before treatments were begun.
[b] One application washed off.
[c] Two applications washed off; one application of DDT to all plots for bollworms not included.
[d] Does not include two applications of DDT to all plots for bollworms.
[e] Two applications washed off in one plot.

phosphorodithioate) plus DDT, Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate), Bayer 29493 (*O,O*-dimethyl *O*-[4-methylthio-*m*-tolyl] phosphorothioate), barthrin, General Chemicals 3707 (1,3-bis(methoxycarbonyl)-1-propen-2-yl dimethyl phosphate, Monsanto CP-7769 (hexaethyl (ethylthiomethylidyne)triphosphonate), and BHC plus DDT. The large-plot experiment included two dust treatments, Sevin and Guthion plus DDT, replicated two times in the same field. The plots ranged from 0.29 to 1.04 acres in size.

Sulfur was incorporated in all the dusts, except the mixture containing Guthion, to suppress spider mite infestation.

The insecticide applications were scheduled at weekly intervals. Rotary hand guns and a self-propelled sprayer that could cover 12 rows were used in the small plots, whereas both hand guns and tractor dusters were employed in the large plots. Applications washed off by rain within 24 hours were repeated as soon as conditions permitted.

Infestation records were made for the pink bollworm, boll weevil, bollworm, and cotton fleahopper. However, the pink bollworm was the only insect causing an infestation of primary importance in each test. Five to seven pink bollworm infestation counts were made in the different tests after treatments started. The infestation counts for the other insects ranged from one to eight for the several experiments. Cotton yield records were obtained in all experiments.

Results.—A summary of the insecticide applications, infestation records, and yields is given in table 1. A high infestation of pink bollworm occurred in all the tests at the time the first treatment was applied, or during the first week after the cotton had begun to bloom. Sevin at 10% and the mixture of 2.5% Guthion and 5% DDT were both very effective against the pink bollworm in all tests. The results were noticeably better for both treatments in the large plots than in the small plots. The increases in yield of seed cotton per acre for the Guthion-DDT mixture and the 10% Sevin were 1,845 and 1,773 pounds, respectively, for the large-scale tests, as compared with 1,158 and 1,248 pounds in the small plots. The 5% Sevin was less effective than the 10% Sevin against the pink bollworm.

A mixture of BHC and DDT gave satisfactory control of both the pink bollworm and the bollworm. Bayer 29493 and 25141 were both effective against the pink bollworm but ineffective against the bollworm. Barthrin showed

some promise in controlling the pink bollworm. General Chemicals 3707 and Monsanto CP-7769 were not effective against any of the aforementioned insects.

Cotton fleahopper infestation was too low for determining the effectiveness of the insecticides against this insect.

As indicated by the infestation records, the pink bollworm was the major insect causing a reduction in cotton yield; however, the bollworm did affect the yields in some of the small-plot experiments.

Early-Season Thrips Control on Cotton in New Mexico[1]

S. R. RACE, *New Mexico Agricultural Experiment Station, University Park*

ABSTRACT

In-furrow planting applications of Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and phorate controlled heavy infestations of western flower thrips, *Frankliniella occidentalis* (Pergande) on cotton seedlings for 7 and 8 weeks, respectively. Spray applications of methyl parathion and methyl parathion plus toxaphene also controlled thrips, but toxaphene alone did not.

Di-Syston and phorate applied as side dressings to cotton plants 9 weeks after planting did not significantly reduce populations of lygus bugs, *Lygus lineolaris* (Palisot de Beauvois), *L. hesperus* Knight and *L. elisus* Van Duzee, and cotton fleahoppers, *Spanogonicus albofasciacus* Reuter.

Plant stands were not significantly affected by the treatments although yield data indicate that fruiting and plant maturation were delayed by the phorate treatments.

The results showed that early-season thrips control on cotton in New Mexico significantly increased yields, and that in-furrow applications of Di-Syston were especially effective.

The control of thrips on cotton seedlings with the systemic insecticides phorate and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) has been reported by several workers. Ivy et al. (1957) noted that an activated carbon-phorate coating on cotton seed effectively reduced onion thrips, *Thrips tabaci* Lindeman, and flower thrips, *Frankliniella tritici* (Fitch), for 7 and 8 weeks, respectively. Parencia et al. (1957) showed that thrips (*Frankliniella* sp.) were controlled for 3 to 4 weeks after seedling emergence with various concentrations of both carbon phorate and Di-Syston but that the cotyledons showed phytotoxic symptoms. Hanna (1958) observed that cotton plants grown from seed treated with phorate and Di-Syston were protected from tobacco thrips, *F. fusca* (Hinds), and *F. tritici* 4 to 5 weeks after planting and received another 2 weeks of variable control. He also noted some reduction in stand as well as slower fruiting and maturation of plants which had been seed treated. Hopkins et al. (1958) showed that seed treatments of phorate and Di-Syston adversely affected stands but that in-furrow applications of phorate granules did not.

In New Mexico, the western flower thrips, *Frankliniella occidentalis* (Pergande), is usually a serious pest of cotton during the early growing season. Heavy infestations severely crinkle the cotyledons and in many cases kill the young terminals, a serious condition under the limited New Mexico growing season. Consequently, many New Mexico growers plan on two and possibly three post-emergence sprays as soon as thrips populations average two per seedling. Thus, a preventive or insurance thrips control program would benefit the cotton growers.

However, due to the conflicting reports concerning reduction in plant stands, delayed fruiting, and the lack of evidence showing increased yields (Dobson 1958), such carbon seed treatments were deemed inadvisable for general usage. Since the work of Hopkins and co-workers indicated no adverse stands due to in-furrow applications of granules, an experiment designed to compare the effects of granulated systemic insecticides with a postemergence spray treatment on thrips populations and cotton production was conducted near Hatch, New Mexico during 1960.

METHODS.—On April 7, acid-delinted, Acala 1517C cotton seed (treated with 2 oz. actual Ceresan per 100 lb.) was planted at the rate of 20 pounds per acre. Granulated insecticides were discharged into the seed furrows with the seed and immediately covered with soil. The flow of granules was metered by two 2-row distributors[2] regulated to apply 1 pound of actual insecticide per acre (10 pounds of 10% granules per 13,756 feet of row). At planting time, the soil temperature was approximately 10° C. and the soil moisture caused by the pre-plant irrigation was adequate for plant growth until the first or second week in June. Each spray treatment was applied with a conventional four-row boom type spray rig.

Additional applications of phorate and Di-Syston were made to certain plots on June 9 immediately before the first irrigation. This was done to measure their effects on the populations of injurious mid-season insects such as lygus bugs, *Lygus lineolaris* (Palisot de Beauvois), *L. hesperus* Knight and *L. elisus* Van Duzee, and a cotton fleahopper, *Spanogonicus albofasciatus* Reuter. These applications were made by adjusting the distributor[2] so as to discharge the granules immediately behind two chisels mounted on a small tractor. Each chisel cut a furrow 4″ deep or approximately at the original seed level and 6″ to the side of the plants. One side of each row was treated. Both the phorate and Di-Syston were applied at the rate of 30 pounds of 10% granules per acre (3 pounds of actual insecticide per acre). One series of the above plots received a planting application of systemic insecticide while a second had received none.

The treatments included in this experiment were the following:

[1] Journal series No. 165, New Mexico Agricultural Experiment Station, University Park, New Mexico. Accepted for publication April 18, 1961.

[2] "Gandy" units—Model No. 901-2 manufactured by the Gandy Company, Inc., Owatonna, Minnesota.

Insecticide Treatments:

Planting Application April 7, 1960	*First Irrigation Application June 9, 1960*
1. Di-Syston (1 lb./acre)	+No treatment
2. Di-Syston (1 lb./acre)	+Di-Syston (3 lb./acre)
3. No treatment	+Di-Syston (3 lb./acre)
4. Phorate (1 lb./acre)	+No treatment
5. Phorate (1 lb./acre)	+Phorate (3 lb./acre)
6. No treatment	+Phorate (3 lb./acre)

Insecticide Spray Treatments:

7. Spray schedule No. 1[3]
8. Spray schedule No. 2[4]

Untreated Check:

9. Untreated

In past years toxaphene sprays have been commonly used for early-season cotton insect control in the Hatch Valley and were therefore included in the present study. The first two toxaphene sprays (April 22 and 29) failed to reduce the thrips populations consequently two methyl-parathion sprays were substituted (May 6 and 10). On May 23 a toxaphene + methyl parathion spray was applied to control thrips as well as lygus bugs and fleahoppers.

Each plot was eight rows wide and each row was 1,000 feet long (0.58 acre). The treatments were randomized and replicated four times.

The thrips were collected by quickly placing 10 randomly selected seedlings from each plot into 1-quart cartons for processing in the laboratory. Essentially, the laboratory process involved placing the 10 seedlings from each plot into separate modified Berlese funnels and driving the thrips from the plants into a small vial of alcohol by means of an irritant (methyl isobutyl ketone). A stereomicroscope was used to determine the numbers of larval and adult thrips in each sample.

Counts of the stand were taken from 50 feet of row in the center of each plot. Counts of bloom were made on July 16, 20, and 25, by counting the number of full-blown blossoms in these same rows.

The weights of seed cotton were obtained by mechanically harvesting the entire eight rows of each plot. The first picking took place on October 21 and the second on November 15.

RESULTS.—*Insect Control.*—Plant emergence was good and an excellent stand was obtained in 9 days. A cool dry period followed and lasted between 3 and 4 weeks during which time the abundant thrips population increased with unusual rapidity. Approximately 5 weeks after planting, the weather became milder and the thrips population declined and remained comparatively low, as shown in table 1. Heavy populations of thrips were controlled in the phorate + no treatment plots up to 8 weeks after planting and for 7 weeks in the Di-Syston + no treatment plots (table 1). (Thrips counts from the phorate + phorate plots and the Di-Syston + Di-Syston plots, which received similar planting treatments as the phorate + no treatment and Di-Syston + no treatment plots, respectively, are not included in this table since experimentally they were assumed to be the same.) The toxaphene sprays in the Spray 1 plots failed to control thrips and two applications of methyl parathion were required to bring the infestation under control. The untreated plots were heavily infested until May 9 when the thrips abundance declined in general.

The condition of the seedlings during the peak of the thrips infestation varied considerably owing to the treatments. The in-furrow phorate-treated plants grew vigorously and had no crinkled leaves. These seedlings were observed to pass through the 4-, 6-, and 8-leaf stages sooner than the Spray 1 and untreated plants. The in-furrow Di-Syston-treated plants grew less vigorously than the phorate-treated plants and showed some leaf crinkling. Both phorate and Di-Syston caused necrotic lesions on the outer margins of the cotyledons, which the other treatments did not. The Spray 1 plots commenced vigorous growth only after two applications of methyl parathion spray. Growth was surpressed in the untreated plots (no treatment + phorate, no treatment + Di-Syston, Spray 2, and check) until early June.

Data on the insect collections from each of the plots receiving sidedressing applications of systemic insecticide were extremely variable, and no significant differences in lygus bug and fleahopper populations were noted.

Stand Counts.—Counts made during April, May, and early June showed no significant differences in plant stand

[3] Applications as follows: April 22—1.7 lb. toxaphene/acre; April 29—1.7 lb. toxaphene/acre; May 6—0.12 lb. methyl parathion/acre; May 10—0.12 lb. methyl parathion/acre; May 23—0.25 lb. methyl parathion + 1.7 lb. toxaphene/acre; May 31—1.7 lb. toxaphene/acre; June 7—1.7 lb. toxaphene/, acre.

[4] Same as Spray No. 1 except the first four applications were omitted.

Table 1.—The influence of insecticides on the population of *Frankliniella occidentalis* on cotton seedlings, Hatch, New Mexico, 1960.[a]

TREATMENT	APRIL					MAY								JUNE		SEASONAL AVERAGE
	20	22	23	27	30	3	7	9	11	14	19	21	25	1	6	
Di-Syston + No treatment	0	0.7	—	0.6	3.3	1.7	6.6	2.3	—	1.7	—	—	4.5	4.2	7.5	3.2
Phorate + No treatment	0	0	—	0.1	0.9	0.3	0.2	0.5	—	0.2	—	—	1.4	2.8	3.6	0.9
Spray 1[b]	—	—	5.5	29.9	23.5	23.5	8.9	6.5	3.1	2.1	5.1	4.5	0.5	1.5	—	9.6
Untreated	0	0.5	6.3	26.4	16.6	21.5	22.7	8.9	9.9	1.8	2.2	2.1	7.5	7.8	6.7	9.9
L.S.D. at 5% level	—	—	—	12.4	6.7	8.4	6.7	3.2	—	n.s.	—	—	2.9	3.7	n.s.	
at 1% level	—	—	—	17.8	9.6	12.1	9.7	4.6	—		—	—	3.9	5.3		

[a] Each figure represents the average number of larvae per seedling.

[b] Sprayed April 22 and 29 and May 31 with toxaphene—1.7 lb./acre; May 6 and 10 with methyl-parathion —0.12 lbs. /acre; May 23 with toxaphene—1.7 lb. + methyl parathion—0.25 lb./acre.

Table 2.—The influence of insecticides on the final stand of cotton June 9, 1960. Hatch, New Mexico.

TREATMENT	SEEDLINGS PER 50 FEET OF ROW[a]
Di-Syston+ No treatment	167
Di-Syston+Di-Syston	157
No treatment+Di-Syston	153
Phorate+No treatment	142
Phorate+Phorate	141
No treatment+Phorate	156
Spray 1	167
Spray 2	174
Check	148
L.S.D. at 5% level	n.s.

[a] Average of four replications.

owing to treatment. None of the cotton plants were thinned in any of the experimental plots. Results of the final stand count, taken on June 9, are presented in table 2.

Bloom Counts.—The results of the cotton bloom counts are presented in table 3. Bloom counts in the in-furrow phorate-treated plots, although not significantly different from those in the untreated plots on any of the sampling dates, are decidedly the lowest in number of all the treatments.

Yield.—Table 4 presents the yields of seed cotton from the first and second harvests. The first picking shows no significant increases in yields between any of the treatments and the untreated plots. The no treatment+Di-Syston, phorate+phorate, phorate+no treatment, Spray 1 and Spray 2 treatments yielded less cotton at first picking than the untreated check. At the second picking each treatment yielded significantly more cotton than the check. The increases are especially noticeable in the in-furrow phorate treatments, where the fruiting was most delayed. The total yield from each treatment, although not significant in each case, was higher than that of the untreated.

SUMMARY AND DISCUSSION.—Granulated Di-Syston and phorate applied in the seed furrow at planting time controlled *Frankliniella occidentalis* on cotton plants up to 7 and 8 weeks after planting. Sprays of methyl parathion alone and methyl parathion plus toxaphene controlled similar thrips infestations, but toxaphene alone

Table 3.—The influence of insecticides on cotton blooms per 50 feet of row. Hatch, New Mexico. 1960.

TREATMENT	TREATMENT MEANS[a]		
	July 16	July 20	July 25
Di-Syston+No treatment	24.2*	31.2	60.5
Di-Syston+Di-Syston	17.7	23.0	49.2
No treatment+Di-Syston	15.5	21.0	55.2
Phorate+No treatment	8.2	17.5	41.5
Phorate+Phorate	10.2	19.2	39.0
No treatment+Phorate	19.7	24.7	62.5
Spray 1	14.5	15.5	51.2
Spray 2	10.2	21.5	50.7
Untreated	14.7	22.5	53.7
L.S.D. at 5% level	8.7	n.s.	n.s.
at 1% level	11.8		

[a] Average of four replications.

Table 4.—The influence of insecticides on pounds of seed cotton per acre.[a] Hatch, New Mexico, 1960.

TREATMENT	POUNDS			
	1st Picking	2nd Picking	Total	Increase Over Check
Di-Syston+No treatment	2,706	957**	3,663**	461
Di-Syston+Di-Syston	2,616	1,065**	3,681**	479
No treatment+Di-Syston	2,306	991**	3,298	96
Phorate+No treatment	2,462	1,195**	3,657**	455
Phorate+Phorate	2,255	1,229**	3,484	282
No treatment+Phorate	2,557	936*	3,493*	291
Spray 1	2,531	918*	3,450	248
Spray 2	2,429	979**	2,408	206
Check	2,542	660	3,202	—
L.S.D. at 5% level	231	216	289	
at 1% level	313	293	392	

[a] Each figure represents the average of four replications.

failed in this respect.

Comparisons between the granulated systemic treatments and the postemergence spray treatments are limited due to the complex of insecticides applied to each of the spray plots.

Stands of plant were not affected by the treatments with granulated materials as they apparently have been in New Mexico when the seed treatments with carbon formulations were used (Dobson 1958). However, the physiology of the plants was apparently altered enough by the phorate treatments to delay fruiting and maturation. This fact was manifested in the reduced yields of the first picking from these plots and the highly significant increases in yield from the second picking. Di-Syston caused no such apparent interference in the normal development of the cotton plants.

Lygus bugs and fleahoppers were not significantly controlled by sidedressings of Di-Syston or phorate, although the treatments were apparently not without some influence since increases in yield were obtained.

The results from this experiment show that yields of seed cotton were significantly increased by controlling early season infestations of western flower thrips and that in-furrow applications of granulated Di-Syston were especially effective in this respect.

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Spray Programs to Control Citrus Rust Mite in Florida[1]

ROGER B. JOHNSON, *Florida Citrus Experiment Station, Lake Alfred*

ABSTRACT

Experiments to avoid the constant use of zineb (zinc ethylene-bis-dithiocarbamate) against citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), by integrating 5.2 ounces of zineb per 100 gallons of spray, 2.0 ounces of Chlorobenzilate® (ethyl 4,4′-dichlorobenzilate) and 160.0 ounces of sulfur in spray programs were conducted on Valencia orange trees. Three sprays were used in each program: postbloom, midsummer and fall. Chlorobenzilate was a good substitute for zineb in postbloom sprays, but poor in the summer; sulfur was unsatisfactory in both postbloom and summer sprays; and Chlorobenzilate, zineb and sulfur were equal in the fall. The sequence of postbloom Chlorobenzilate, summer zineb and fall sulfur was a good substitute for three applications of zineb.

Before 1957 sulfur and lime-sulfur were the only materials used in Florida to control citrus rust mite, *Phyllocoptruta oleivora* (Ashmead). In 1957, however, Fisher reported that zineb (zinc ethylene-bis-dithiocarbamate) controlled russeting of citrus fruits and Johnson et al. (1957) showed that zineb and maneb (manganese ethylene-bis-dithiocarbamate) controlled citrus rust mite. Soon many citrus growers abandoned sulfur and this action engendered predictions that intensive use of zineb would produce resistant mites or new problems. One solution to these anticipated problems was to alternate zineb with other effective rust mite acaricides and experiments were started to determine the best integration of sulfur, zineb and Chlorobenzilate® (ethyl 4,4′-dichlorobenzilate) (Jeppson et al. 1955) in a spray program. The aim of this program was not to eliminate citrus rust mite with each spray, but to prevent economic damage to trees and fruit by suppressing seasonal increases in the mite population.

MATERIALS AND METHODS.—Four experiments were conducted in Valencia orange groves near Lake Alfred, Florida. Sprays in each test were applied when the petals had dropped, in midsummer and in the fall. This number and timing of applications was selected as the minimum required to control the important pests and diseases in most Florida citrus groves: the postbloom spray to control diseases and citrus rust mite, the summer spray for scale insects and citrus rust mite and the fall spray for spider mites and citrus rust mite. Summer and fall sprays were delayed, however, until about 20% of the leaves on trees sprayed with zineb were infested with citrus rust mite. This timing assured that sulfur and Chlorobenzilate would usually be applied to higher populations.

A conventional hydraulic sprayer equipped with two double Boyce guns and operated at 600 p.s.i. at the pump was used. Applications were made when there was little air movement and applied to the point of run-off with no effort made to cover inside limbs and foliage except where parathion was included to control scale insects.

Treatments were evaluated for effectiveness against citrus rust mite by frequent determinations of the density of mite populations in the manner described by Johnson (1958). Intervals of control were arbitrarily set at the number of weeks between the date of application and the date when 20% of the leaves were reinfested.

Plots of four trees were randomized in three blocks in Experiments A and C, and four trees in four blocks in Experiments B and D. Spray programs and dosages are shown in tables 1, 2, and 4. The dosage of each material was the most economically effective amount used on Florida citrus. Three applications of zineb was the standard program and check plots were provided in all experiments. In Experiments A, C, and D, 1.0 lb. of 25% parathion per 100 gallons of spray was used to control scale insects in the summer application while 0.6 lb. was used in both postbloom and summer sprays in Experiment B. One-half lb. of 25% Tedion® (*p*-chlorophenyl 2,4,5-trichlorophenyl sulphone) was included in all fall sprays to control citrus red mite, *Panonychus citri* (McGregor), and Texas citrus mite, *Eutetranychus banksi* (McGregor).

RESULTS.—*1958.*—Data in tables 1 and 2 show that three applications of zineb gave the best control of citrus rust mite in Experiments A and B. Programs with Chlorobenzilate and especially sulfur were less effective: postbloom zineb sprays controlled citrus rust mite 2 weeks longer than Chlorobenzilate and 4 weeks longer than sulfur while summer control with zineb lasted 2 to 6 weeks longer than Chlorobenzilate and 12 weeks longer than

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Table 1.—Control of citrus rust mite with spray programs used in Experiment A, 1958.

Active Ingredient of Acaricides (oz./100 gal.) Applied on Date Shown			Mean Number of Leaves Infested with Citrus Rust Mite After Each and All Applications			
April 23	July 12	October 8	May to July	July to Oct.	Oct. to Mar.	May to Mar.[a]
Unsprayed	Unsprayed	Unsprayed	74	39	33	45.5 e
Zineb, 5.2	Zineb, 5.2	Zineb, 5.2	14	4	17	11.9 a
Sulfur, 160.0	Zineb, 5.2	Zineb, 5.2	38	3	21	19.3 c
Sulfur, 160.0	Sulfur, 80.0	Zineb, 5.2	36	39	26	32.6 d
Zineb, 5.2	Zineb, 5.2	Sulfur, 160.0	7	4	33	16.9 bc
Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	28	10	14	16.3 b

[a] Results of Duncan's Test: treatment means followed by different letters are significantly different at the 1% level.

Table 2.—Control of citrus rust mite with spray programs used in Experiment B, 1958.

Active Ingredient of Acaricides (oz./100 gal.) Applied on Dates Shown			Mean Number of Leaves Infested with Citrus Rust Mite After Each and All Applications			
April 23	July 9	November 20	May to July	July to Nov.	Dec. to Mar.	May to Mar.[a]
Check			38	33	42	36.9 e
Zineb, 5.2	Zineb, 5.2	Zineb, 5.2	5	9	1	5.4 a
Sulfur, 160.0	Zineb, 5.2	Zineb, 5.2	17	15	0	11.0 b
Sulfur, 160.0	Sulfur, 80.0	Zineb, 5.2	22	42	1	23.7 d
Zineb, 5.2	Zineb, 5.2	Sulfur, 160.0	2	12	1	6.4 a
Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	7	26	0	13.6 c

[a] Results of Duncan's Test: treatment means followed by different letters are significantly different at the 1% level.

Table 3.—Mean number of Valencia oranges infested with citrus rust mite from May to February compared with the number of russet fruit in February.

Active Ingredient of Acaricides (oz./100 gal.)			Experiment A[a]		Experiment B[a]	
Postbloom	Summer	Fall	Infested	Russet	Infested	Russet
Check			35.9 e	99.0 d	23.4 e	59.8 c
Zineb, 5.2	Zineb, 5.2	Zineb, 5.2	4.3 b	5.0 a	1.6 a	4.2 a
Sulfur, 160.0	Zineb, 5.2	Zineb, 5.2	10.4 c	37.9 b	4.6 b	29.5 bc
Sulfur, 160.0	Sulfur, 80.0	Zineb, 5.2	23.2 d	66.8 c	21.2 d	51.2 c
Zineb, 5.2	Zineb, 5.2	Sulfur, 160.0	2.2 a	3.3 a	0.7 a	3.0 a
Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	10.7 c	15.3 ab	8.3 c	16.5 b

[a] Results of Duncan's Test: treatment means followed by different letters are significantly different at the 1% level.

Table 4.—Control of citrus rust mite with spray programs used in Experiments C and D, 1959.

Active Ingredient of Acaricides (oz./100 gal.) Applied on Dates Shown			Mean Number of Leaves Infested with Citrus Rust Mite from April, 1959 to March, 1960[a]	
March 25, 26	June 25, 26	September 29, 30	Experiment C	Experiment D
Unsprayed			26.9 e	21.5 f
Zineb, 5.2	Zineb, 5.2	Zineb, 5.2	5.6 a	1.8 a
Sulfur, 160.0	Zineb, 5.2	Zineb, 5.2	9.4 c	3.1 c
Sulfur, 160.0	Sulfur, 160.0	Zineb, 5.2	14.5 d	9.2 e
Zineb, 5.2	Zineb, 5.2	Sulfur, 160.0	4.6 a	2.3 ab
Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	Chlorobenzilate, 2.0	6.9 b	5.4 d
Chlorobenzilate, 2.0	Zineb, 5.2	Chlorobenzilate, 2.0	5.6 a	3.5 c
Chlorobenzilate, 2.0	Zineb, 5.2	Sulfur, 160.0	4.5 a	2.8 bc

[a] Results of Duncan's Test: treatment means followed by different letters are significantly different at the 1% level.

sulfur. There was no difference between materials in the fall. All materials controlled citrus rust mite from mid-November through March in Experiment B, but in Experiment A the fall spray was applied early in October and control ended in mid-January.

These data show that Chlorobenzilate was the best substitute for zineb in the postbloom spray, only zineb was satisfactory in the summer, and both Chlorobenzilate and sulfur were as good as zineb in the fall. These conclusions are supported by the amounts of russet fruit at harvest in January (table 3).

1959.—Spray programs tested in 1958 were repeated in Experiments C and D and supplemented with Chlorobenzilate-zineb-Chlorobenzilate and Chlorobenzilate-zineb-sulfur programs in 1959. Experiments C and D verified the results of Experiments A and B. Zineb was again superior to Chlorobenzilate and especially sulfur with the greatest differences between materials occurring after the summer application. Differences were less pronounced after the postbloom spray and were insignificant in the fall. Data in table 4 show that zineb-zineb-sulfur; Chlorobenzilate-zineb-sulfur; and Chlorobenzilate-zineb-Chlorobenzilate programs were good substitutes for three applications of zineb.

Discussion.—Yothers & Mason (1930) showed that the number of citrus rust mites usually declines after December; increases in March and April; increases most rapidly in May and June and is most abundant in late June and early July. They further showed a decline from the summer peak to October followed by an increase to a secondary peak in December. This seasonal history indicated that control should be most difficult in the spring and summer when a great increase occurs, but comparatively easy in the fall and winter. The present experiments verified these assumptions. Zineb was slightly better than Chlorobenzilate in postbloom sprays, far superior to Chlorobenzilate and especially sulfur in the summer, but no better than either material in the fall.

These facts indicate that zineb and equal or better materials are best used in postbloom and summer sprays, but that Chlorobenzilate and materials with equal effectiveness may be used in postbloom sprays with only a slight chance that respraying will be needed before July. These facts further indicate that sulfur should be used in the fall and winter or when only a few weeks of control is needed.

These experiments thus show that a spray program of postbloom Chlorobenzilate, summer zineb and fall sulfur will give commercially acceptable control of citrus rust mite under most conditions, but that a fourth spray may occasionally be needed.

The sequence of Chlorobenzilate, zineb, and sulfur has several advantages; it eliminates postbloom mixtures of zineb and copper compounds which may be less effective than zineb alone (Johnson et al. 1957, Johnson 1959, 1960), it permits the use of oil in postbloom and summer sprays without a compatibility problem with sulfur, and places Chlorobenzilate in the spring and sulfur in the fall and winter when it may be necessary to control *Brevipalpus* sp. (Knorr & Thompson 1954).

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Preferred Feeding and Egg Laying Sites of the Boll Weevil and the Effect of Weevil Damage on the Cotton Plant[1]

E. P. LLOYD,[2] J. L. MCMEANS,[3] and M. E. MERKL[2,4]

ABSTRACT

During 1958 studies of the preferred feeding and egg-laying sites of the boll weevil (*Anthonomus grandis* Boheman) were conducted at Stoneville, Miss. Females damaged the fruit of the cotton plant more than males. The preferred feeding and egg-laying sites were the squares on the upper half of the plants when the population was low. As the population increased, small bolls were damaged as well as squares; the damage was intensified on the upper portions of the plants and reached into the lower fruiting branches. Bolls from 1 to 19 days old were subject to weevil damage.

Exposure of the cotton plant to feeding and egg laying by the boll weevil for 4 or 5 days resulted initially in increased square abscission and slightly reduced boll abscission. No difference occurred in total boll set for the entire season between the test and check plants.

It has long been known that the boll weevil (*Anthonomus grandis* Boheman) has a preference for the squares (flower buds) of the cotton plant. Townsend (1895) stated that female weevils deposited their eggs first in buds until all were infested and then oviposited in the smallest bolls. Hunter (1912) observed that squares were greatly preferred as food and as places for depositing eggs. In an experiment in which squares and bolls of sea-island and upland cotton were removed from the plants and confined with gravid females, Howard (1921) showed that squares were the preferred place for oviposition. Fenton & Dunnam (1929) observed that weevils most frequently deposited eggs in squares from the time the squares were 6 days old until 3 days prior to bloom. In squares 3 days or less from blooming, feeding punctures were more numerous.

Several workers have simulated boll weevil damage by mechanical removal of squares from the plant. The effect of this damage on the plant was then observed and measured, usually in terms of final yield of seed cotton. Ewing (1918) and Hamner (1943) showed that the mechanical removal of squares improved subsequent boll set. Hamner showed that a complete loss of young squares through the third week in July did not reduce the yield of seed cotton if plants were protected from further damage by insects.

Valentine (1958) found that cotton plants were able to recover and produce a crop without loss in yield even after being subjected to 4 weeks of bollworm damage.

An investigation was begun in 1957 at the Delta Branch Experiment Station at Stoneville, Miss., to determine responses of the cotton plant to different boll weevil populations when high soil fertility and adequate moisture were provided. Preliminary experiments were conducted during 1957 to establish methods to determine the sites on the plant preferred by the boll weevil for feeding and oviposition and the effect of damage caused by these activities on the fruiting of the cotton. Results of experi-

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[2] Entomologists, Entomology Research Division, Agric. Res. Serv., U.S.D.A.
[3] Plant Physiologist, Crops Research Division, Agric. Res. Serv., U.S.D.A.
[4] In cooperation with the Mississippi Agricultural Experiment Station.

Table 1.—Average damage by different boll weevil populations confined for 4- or 5-day periods to individual cotton plants.

Population	No. of Squares Damaged	Square Abscission (%)	No. of Bolls Damaged	Boll Abscission (%)	Number of Punctures	
					Feeding	Egg
Tests Run on Aug. 11–15, 15–20, 20–25						
1 Male	3.5	29	—	—	5.6	—
1 Female	10.6	80	0.1	0	14.7	9.6
1 Pair	12.3	80	1.1	11	16.1	9.0
Tests Run on Aug. 25–29, Aug. 29–Sept. 3, Sept. 3–8						
2 Pairs	6.3	66	2.1	40	19.0	4.9
4 Pairs	14.2	71	4.0	28	41.0	9.2
6 Pairs	22.2	65	5.4	22	66.3	17.7
Tests Run on Sept. 8–12, 12–19, 19–24						
8 Pairs	18.8	78	14.2	55	108.2	18.6
12 Pairs	19.0	89	12.8	56	115.7	13.7
16 Pairs	23.0	86	18.0	36	146.6	22.5

ments conducted during the 1958 growing season are presented.

METHODS AND MATERIALS.—Cotton seed, variety Delfos 7343, was planted on May 26, 1958. Nitrogen was applied to the soil at the rate of 100 pounds per acre. Prior to first bloom plants were thinned to 1 per 6 feet of row. To facilitate the interpretation of data all vegetative and secondary fruiting branches were removed at weekly intervals so that each plant consisted only of the main axis and primary fruiting branches.

Boll weevil population levels used in these experiments were as follows: 1 male; 1 female; and 1, 2, 4, 6, 8, 12 and 16 pairs. On August 11 one plant each was caged for a 4-day period in each of nine plastic-screen field cages 6 feet on a side. Check plants were not caged. Eight other successive cagings were made on individual plants for 4 or 5 days, with a total of 108 plants being caged. Each caging included three weevil population levels and a check with no weevils and all were replicated three times. These population levels were repeated three times; thus a total of nine replicates for each population level was included. Table 1 gives the population level and caging date for each test.

At the end of each period, cages were removed from the plants, live weevils recovered, and the damage to the squares or bolls recorded. The plants were protected from insect damage with twice-weekly applications of methyl parathion-DDT, prior to and following caging. Plants to be caged were not subjected to insecticide treatment for 5 days prior to caging. To insure that plants contained no damaged forms, all were inspected prior to placing cages over them.

On October 7 all plants were removed from the field and dried. When all bolls had opened, diagrams were made of the individual plants. Data recorded included date of flowering for all bolls; location of squares at the time of weevil confinement; location, nature, and extent of damage on individual forms (flower buds, flowers, bolls); and the fate of these forms.

RESULTS AND DISCUSSION.—*Preferred Feeding and Egg-Laying Sites.*—The damage caused by the different weevil populations while confined on individual plants for 4- or 5-day periods is shown in table 1. Individual males damaged an average of 3.5 squares per plant, whereas a single female damaged 10.6 squares. One pair of weevils caused damage very similar to that of a single female. Four pairs of weevils during late August and early September damaged an average of 14.2 squares and 4.0 bolls per plant. The average number of squares damaged by 6, 8, 12, and 16 pairs of weevils per plant was similar to that of the lower populations. However, the damage on individual squares increased as the weevil population increased.

Each male weevil caused a 29% abscission of squares, whereas each female caused 80%. The average rate of square abscission for all populations, excluding the one-male-per-plant population, was 78.4% and ranged from 65% to 89%.

Feeding punctures per plant increased with the weevil population. However, the number of eggs deposited by 6, 8, 12, and 16 pairs of weevils per plant was similar (table 1). Feeding punctures during the 4- or 5-day caging peri-

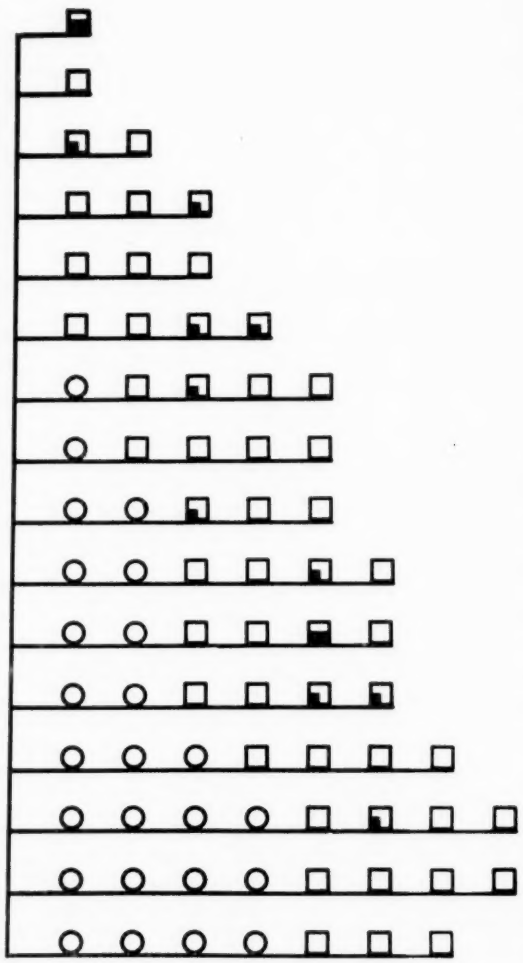

FIG. 1.—Location of damage on the cotton plant caused by a weevil population of 1 male per plant. (Square symbols and circles represent cotton squares and bolls, respectively, whereas the shaded areas show the percentage of replicates damaged at the indicated positions on the plant.)

ods increased progressively from 5.6 for one male to 146.6 when 16 pairs were confined per plant. Females normally deposited only 1 or 2 eggs per square, and at most, 3 or 4 per boll. Results indicated that females were much more selective in choosing sites for depositing eggs than in finding places to feed.

The location on the plants of the forms damaged by one male per plant in mid-August is presented in figure 1. A similar schematic diagram of a cotton plant (fig. 2) shows the location of the damage caused by a population of one female per plant for the same period. Feeding and egg laying were concentrated on the squares on the upper half of the plants. The location of damage caused by one pair of weevils per plant was similar to that caused by a single female. Slight boll damage was recorded in addition to that for the squares on the upper half of the plant.

When four pairs of weevils were caged on each plant in late August and early September (fig. 3), feeding and egg laying increased on the upper portions. Some damage was recorded on the lower fruiting branches.

Six pairs per plant caused damage to squares and small bolls on both upper and lower portions, with a higher percentage on the upper half. Damaged bolls also increased.

Eight pairs caused damage to squares and bolls on the upper and lower fruiting branches; however, the damage was concentrated on the upper two-thirds of the plants (fig. 4). Eight pairs were a large enough population to give maximum damage in a 5-day period. With 12 and 16 pairs, no increase in the number of damaged bolls and squares was observed. The principal difference was the intensification of feeding damage on the upper two-thirds of the plant. The rate of egg deposition remained almost constant in spite of the increased weevil population. Migration of the weevils from the plants to the cage screens occurred prior to the end of the 5-day periods in the two higher populations.

Bolls from 1 to 12 days old were subject to damage during August and the first 15 days of September. During late September, shorter days and decreased temperatures resulted in delayed boll maturity. Under such conditions, bolls as old as 19 days were damaged.

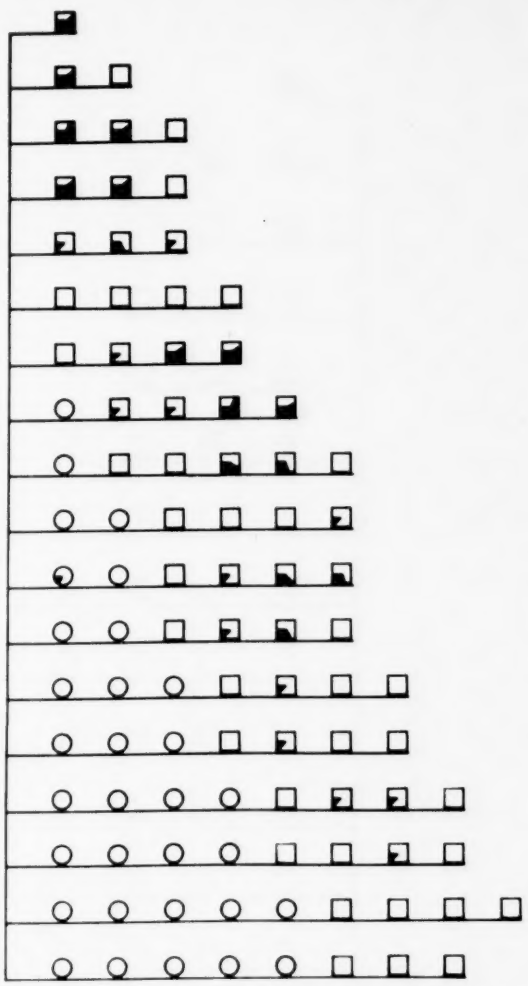

FIG. 2.—Location of damage caused by a weevil population of 1 female per plant. (Square symbols and circles represent cotton squares and bolls, respectively, whereas the shaded areas show the percentage of replicates damaged at the indicated positions on the plant.)

Table 2.—Per cent of cotton forms abscised when caged with different boll weevil populations during entire growing season.

POPULATION	FORMS ABSCISED		TOTAL FORMS ABSCISED
	as Squares	as Bolls	
Total of Tests run August 11, 15, and 20			
1 Male	17	30	47
1 Female	27	27	54
1 Pair	26	25	51
Check	22	26	48
Total of Tests run August 25, 29, and September 3			
2 Pairs	21	28	49
4 Pairs	27	25	52
6 Pairs	28	24	52
Check	21	26	47
Total of Tests run September 8, 13, and 19			
8 Pairs	30	28	58
12 Pairs	28	29	57
16 Pairs	27	30	57
Check	25	29	54

Effect on Fruiting.—Considerable differences existed among plants in the squares and bolls present at the time the tests were initiated. As a result of natural mortality, all weevils were not recovered at the termination of many tests; thus the number of weevils causing the recorded damage was not always consistent within caging periods or replications. In tests initiated on September 13 and 19, migration of weevils from the plants to the cage screens occurred. Even so, definite trends were evident.

In general, 4- or 5-day exposures of the plants to weevils resulted in increased square abscission but reduced abscission of bolls developing from squares that bloomed during this period. These results agreed with the data of Ewing

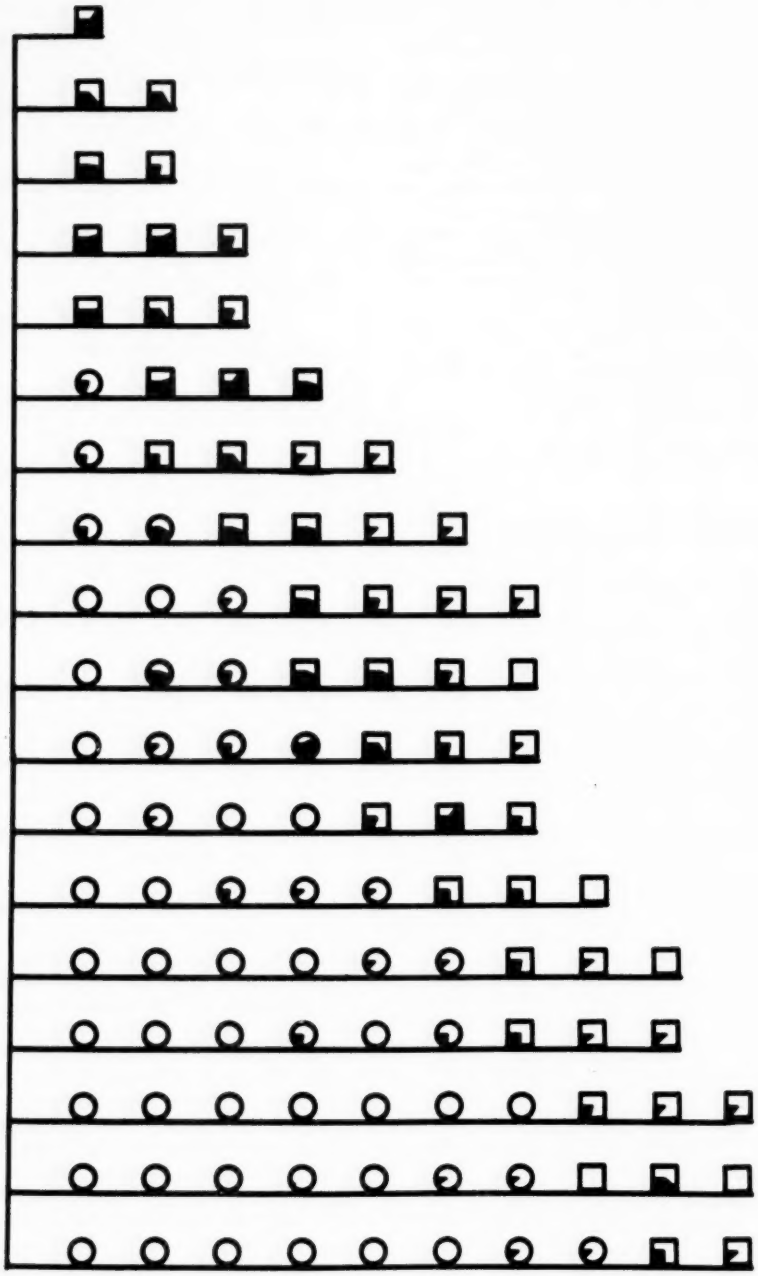

FIG. 3.—Location of damage caused by a weevil population of 4 pairs per plant. (Square symbols and circles represent cotton squares and bolls, respectively, whereas the shaded areas show the percentage of replicates damaged at the indicated positions on the plant.)

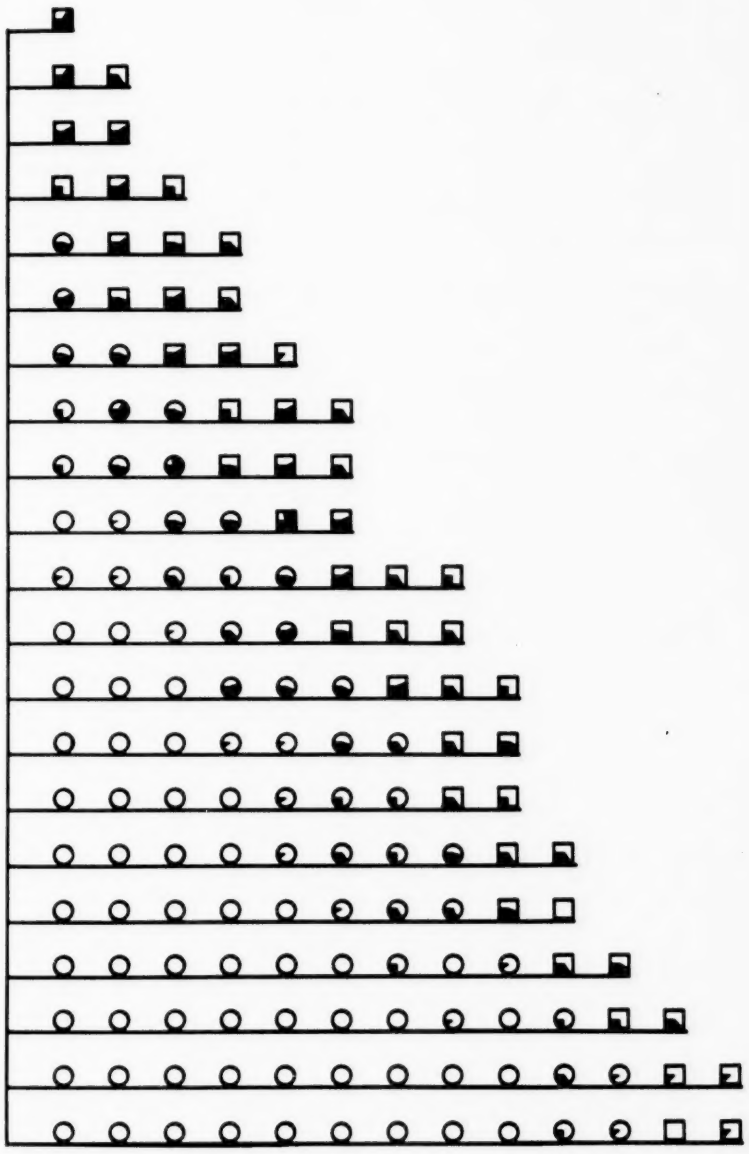

FIG. 4.—Location of damage caused by 8 pairs of weevils per plant. (Square symbols and circles represent cotton squares and bolls, respectively, whereas the shaded areas show the percentage of replicates damaged at the indicated positions on the plant.)

Table 3.—Effect of different boll weevil populations on boll set of cotton plants during entire growing season.

Population	Number of Blooms	Bolls Abscised	Bolls Set	Per Cent of Boll Set
Total of Tests run August 11, 15, and 20				
1 Male	828	304	524	63
1 Female	669	242	427	63
1 Pair	802	276	526	65
Check	633	212	421	66
Total of Tests run August 25, 29, and September 3				
2 Pairs	741	260	482	65
4 Pairs	740	259	481	65
6 Pairs	793	279	514	64
Check	773	266	507	65
Total of Tests run September 8, 13 and 19				
8 Pairs	810	358	452	55
12 Pairs	826	353	473	57
16 Pairs	863	355	508	58
Check	898	401	497	55

(1918) and Hamner (1943), that the mechanical removal of squares improved subsequent boll set. The increased square abscission and reduced boll abscission at the time of exposure became less pronounced as the growing season advanced, and were not evident at the termination of the tests (table 2).

No difference occurred in boll set for the entire growing season as shown in table 3. This result agreed with the data of Valentine (1958), whose tests showed that plants exposed to insect damage for periods up to 4 weeks had no loss of yield.

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Effects of Gibberellin on Nutrition of the Mites, *Tetranychus telarius* and *Panonychus ulmi*[1]

J. G. Rodriguez and J. Milton Campbell,[2] *University of Kentucky, Lexington*

Abstract

Dwarf Horticultural beans and Delta Pine cotton grown in artificial culture in the greenhouse were treated with gibberellin at the rates of 1, 2 and 3 micrograms per plant. Close apple trees grown in semi-field conditions were sprayed with an emulsifiable concentrate of gibberellin at 7- to 10-day intervals during the season at the rates of 10, 50, 100, 250 and 500 p.p.m. in the spray solution. *Tetranychus telarius* (L.) were cultured on detached leaves from all three host plants, and *Panonychus ulmi* (Koch) were cultured on leaves from the apple trees. Gibberellin treatments increased plant height in beans and cotton and total branch length in apple trees and resulted in reduced populations of *T. telarius* in beans and apple trees. Effects of gibberellin on mites cultured on cotton leaves were variable. *P. ulmi* populations were increased by 10 p.p.m. treatment on apple trees but were reduced as were *T. telarius* on the higher treatments and were correlated positively and significantly with per cent total sugars in the apple leaves. Both species were generally positively related to per cent nitrogen, which was decreased in the apple leaves by gibberellin treatments. Reducing sugar was not significantly correlated with populations of either mite species. It is suggested that there is an optimum level of reducing sugar for mite development and the percentages in these host plants fell below the level.

In connection with the mite nutrition investigations which have been conducted in this laboratory over the past several years and with recognition of the general interest in the effects of growth regulators on plants, it was deemed important to treat several common host plants of the two-spotted spider mite, *Tetranychus telarius* (L.), with gibberellic acid in an effort to determine its possible effects on mite development.

Although the literature abounds in reports concerning gibberellic acid and such horticultural characteristics as plant growth, flowering and fruit setting in various types of plants, a paucity of information exists with respect to plant composition and pest response to the hormone. Stowe & Yamaki's review (1957) quotes early Japanese workers to the effect that "diseased" rice plants (affected with gibberellic acid-producing fungus) contained less carbohydrate (total sugars, dextrin, reducing sugar and sucrose) than healthy plants. On the other hand, British workers reported increases in sucrose, fructose and glucose in treated wheat, increased glucose only in peas. Stowe

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director. Accepted for publication April 20, 1961.

[2] Associate Professor and Graduate Student, respectively.

and Yamaki further stated that "nitrogen levels showed inconsistent fluctuations, but these make it unlikely any major increase in protein occurs"; total dry weights may increase or decrease or show no consistent effect; no strong trends could be discerned in ash, potassium or phosphorus. The mineral composition and per cent dry matter of Kentucky bluegrass were not significantly altered on gibberellin treatments, although total sugars were significantly decreased (Wittwer et al. 1957). Foliar application of gibberellin significantly increased the concentration of nitrogen and decreased the concentrations of calcium and boron in the leaves of Montmorency cherry trees (Lewis & Hull 1960); percentages of phosphorus, potassium, magnesium, manganese, iron and copper were not changed.

Eichmeier & Guyer (1960) have evaluated the rate of reproduction of *Tetranychus telarius* reared on Tendergreen snap beans treated with gibberellin. Significant reductions in mite populations occurred on plants receiving certain treatments. There was a reduction in populations on discs cut from treated plants before a significant growth response was shown and the authors concluded that factors other than the growth response of gibberellin-treated plants were associated with the decreased reproduction. The decreased reproductive rate was not permanent, as the rate rose rapidly when the mites were returned to untreated leaves.

The present paper constitutes a report of experiments evaluating the development of *T. telarius* populations cultured on foliage from gibberellic acid-treated apple trees, beans and cotton. Development of the European red mite, *Panonychus ulmi* (Koch), was also studied on the apple trees.

EXPERIMENTAL.—Dwarf Horticultural beans were germinated in silica sand in the greenhouse and the seedlings transferred to artificial culture in the plumule stage. Delta Pine cotton seeds were also germinated in sand and the seedlings transferred to nutrient solutions at the primary leaf stage.

The gibberellic acid solution was prepared by dissolving the powder in a few drops of ethyl alcohol and diluting with water to a concentration of one μg. per drop (0.03 ml.). The solution was applied to the epicotyl, one drop at a time, when the first primary leaves were near full expansion. The plants received 1, 2 or 3 drops at intervals over a period of not more than 24 hours. The cotton plants were also treated when the primary leaves were approximately 1 inch in diameter and before the epicotyl showed.

Close apple trees with a trunk diameter of three-fourth inch at the beginning of the experiments were grown under semi-field conditions. They were set in 30-pound cans filled with Maury silt loam on April 25. Beginning on May 31, they were sprayed at weekly intervals until mid-August with an emulsifiable concentrate (Gibrel®)[3] containing, on the different treatments, 10, 50, 100, 250 and 500 parts per million gibberellin as well as an untreated check. Each treatment included three replicates (trees). Plant growth was measured by extended height in beans and cotton and in the apple trees by measuring the total length of all the branches.

The mites were cultured in detached leaves as described previously (Rodriguez 1953) except that the leaves rested on water-saturated filter paper instead of being floated on a sucrose solution. *Tetranychus telarius* were cultured on all three host plants, *Panonychus ulmi* on apple trees only. Leaves were detached from the first trifoliate of the bean plants and from a comparable rank in the cotton plants. Two series of mite counts, each including four replicates per treatment, were made; each replicate consisted of the progeny developing from five young adult female mites over a developmental period of 8 days. A total of more than 5,500 mites developed on the bean leaves. One series of mite counts, including four replicates per treatment, was made on cotton (a total of 1,700 mites).

On the apple trees, each series of mite counts included three replicates per treatment (one leaf from each tree), each replicate again consisting of the progeny from five females. Six series of *T. telarius* (nearly 8,500 mites) and four series of *P. ulmi* (nearly 6,000 mites) were cultured, the developmental period for the latter species being 10 days.

Analyses for nitrogen, phosphorus and potassium were made on composite samples of apple leaves (one sample from each treatment). Analyses for reducing and total sugars were made separately on the foliage from each tree and from each treatment on beans and cotton. The methods of analysis have been described previously (Rodriguez et al. 1960).

Analyses of variance were performed on the data for plant height and mite populations to determine the variance due to treatment in beans and cotton. In the apple tree data, multiple analyses were performed to determine the variance due to series (or, roughly, the advancement of the season) and the interaction of treatment by series. In cases where the variance due to treatment was significant, the minimal significant difference was calculated. Since the NPK analyses were made on composite samples, analysis for correlation coefficient r between elements and mite populations was based on average populations and not on individual trees.

RESULTS AND DISCUSSION.—*Beans.*—The gibberellic acid treatments, as would be expected, all increased plant height significantly over the check (table 1). The lowest treatment, one μg. of gibberellin, resulted in the development of a *T. telarius* population significantly lower than the check. At the 2 and 3 μg. treatments the mite populations were not significantly different from the check. Eichmeier & Guyer (1960) concluded that their reduction in mite populations on gibberellic-acid-treated beans was

Table 1.—Effect of gibberellic acid on plant height, *T. telarius* populations, and per cent sugar in Dwarf Horticultural beans.

GIBBERELLIN APPLIED TO EPICOTYL (μG.)	PLANT HEIGHT (IN.)	*T. telarius* POPULATION[a]	SUGAR (%)	
			Reducing	Total
0	16.1	151	0.30	1.77
1	34.5	102**	.34	1.56
2	37.9	151	.30	1.64
3	43.8	139	.39	1.32
L.S.D. at 1% level	6.5	41		

[a] Average number of progeny resulting from five female mites cultured for 8 days in detached leaf culture.

[3] Merck potassium salt of gibberellic acid, courtesy Merck and Company.

Table 2.—Effect of gibberellic acid on plant height, *T. telarius* populations and per cent sugar in Delta Pine cotton.

Gibberellin Applied (μG.)	Plant Height (IN.)	*T. telarius* Population[a]	Sugar (%)	
			Reducing	Total
0	10.0	44	0.50	1.10
To epicotyl				
1	12.5	69	.52	1.07
2	13.0	51	.57	.88
3	14.0	56	.61	1.00
To primary leaves				
1	13.3	38	.50	1.87
2	13.8	23	.48	.87
3	12.3	45	.66	1.37

[a] Average number of progeny resulting from five female mites cultured for 8 days in detached leaf culture.

due to some other factor than plant height. Our results confirm this opinion since only one treatment resulted in reduced populations while all three increased plant height. Total sugars were reduced in the treated plants, while no consistent effect was detectable in reducing sugar. This effect on total sugars is consistent with the findings of Wittwer and co-workers (1957) on Kentucky bluegrass. Neither sugar was significantly correlated with mite populations.

Cotton.—The gibberellic acid treatments increased the height of the cotton plants also (table 2). The effect of the treatments on mite populations was variable; some increases and some decreases were noted, none being significant.

Apple Trees.—The total growth of the branches in the untreated apple trees averaged 111 inches per tree. Three gibberellin treatments resulted in growth significantly greater than the check: 10 p.p.m.—176 inches, 250 p.p.m. —229 inches and 500 p.p.m.—227 inches. The branches of the trees treated with 50 p.p.m. and 100 p.p.m. averaged 135 and 149 inches, respectively, which were not

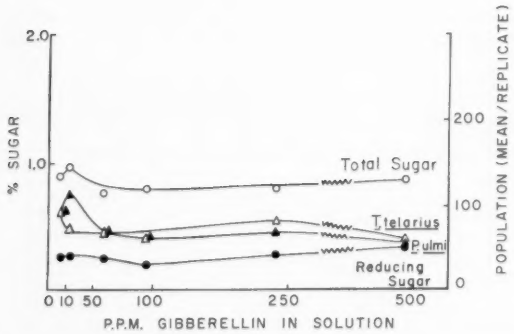

FIG. 1.—Effect of weekly gibberellin sprays on total and reducing sugars and mite populations on foliage of Close apple trees. Mite population is expressed as the average number of progeny resulting from five female mites during a developmental period of 8 days for *T. telarius* (open triangle) or 10 days for *P. ulmi* (solid triangle).

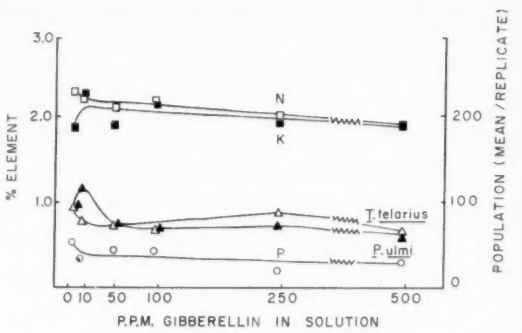

FIG. 2.—Effect of weekly gibberellin sprays on nitrogen, phosphorus and potassium and mite populations on foliage of Close apple trees. Mite population is expressed as the average number of progeny resulting from five female mites during a developmental period of 8 days for *T. telarius* (open triangle) or 10 days for *P. ulmi* (solid triangle).

significantly different from the check. (The minimal significant difference was 42 inches.)

The results of the foliage analyses and mite population counts are shown in figures 1 and 2. The gibberellin treatments decreased the reproductive capacity of *T. telarius*, as evidenced in the populations developing on leaves from trees treated with 50, 100 or 500 p.p.m. gibberellin, which were significantly lower than the check (minimal significant difference = 26 mites). Thus the reduction in population occurs in apple trees as well as beans. *Panonychus ulmi* reacted in a slightly different fashion; a population increase at 10 p.p.m. gibberellin was followed by decreases at the higher rates of treatment, similar to those demonstrated in *T. telarius*. The increase was not significantly different from the check (minimal significant difference = 32 mites); however, populations developing on the untreated and 10 p.p.m.-treated trees were significantly higher than the populations developing on the 500-p.p.m.-treated trees. Thus it was demonstrated that higher concentrations of gibberellin were required to effect a significant reduction in *P. ulmi* than in *T. telarius*.

The gibberellin treatments decreased the percentages of nitrogen, and more erratically phosphorus, in the apple foliage. Both mite species were generally positively related to per cent nitrogen.

Fluctuations in the sugar content of leaves from the individual trees prevented significance between the means, either of reducing or total sugars, although the total sugars varied considerably on the different treatments (fig. 1). *Panonychus ulmi* population was correlated significantly with per cent total sugars ($r = .816^*$). Reducing sugar variations were not significantly correlated with population differences of either mite species.

A previous publication (Rodriguez et al., 1960) showed consistent significant positive correlations between reducing sugar and *T. telarius* populations developing on Black Valentine beans and Clark soybeans. Comparison of those data with the present results revealed that the percentages of reducing sugar in those host plants were considerably higher, ranging generally from 0.5% to 1.3%. The percentages of reducing sugar in beans, cotton and apple trees ranged from 0.30 to 0.39, 0.48 to 0.66 and 0.16 to

0.48, respectively. It appears probable that there is an optimum level of reducing sugar for mite development, as there is an optimum level for other nutrients.

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Dosage Levels and Seasonal Behavior of Insecticides Used in Control of European Pine Shoot Moth[1]

J. W. BUTCHER and R. B. CARLSON,[2] *Michigan State University, East Lansing*

ABSTRACT

In spring and summer hydraulic spray treatments appreciable differences in European pine shoot moth (*Rhyacionia buoliana* (Schiffermüller)) mortality were noted when 0.5, 1.0 and 2.0 pound (per 100 gallon) dosages of DDT, methoxychlor, Sevin® (1-naphthyl-*N*-methylcarbamate) and American Cyanamid SF60 (malathion plus sticker) were used. Excellent control was obtained with 0.5, 1.0 and 2.0 pounds of dimethoate in both spring and summer treatments. Both DDT and methoxychlor proved noticeably less effective in summer than in spring applications, while the opposite was true for Sevin.

In an earlier paper, Butcher & Haynes (1960) reviewed published reports of investigations on direct control of European pine shoot moth, *Rhyacionia buoliana* (Schiffermüller). They concluded that (1) spring applications of insecticides should be delayed until overwintering larvae resumed feeding, and (2) optimum performance of Sevin®(1-naphthyl *N*-methylcarbamate), dimethoate and DDT would likely be achieved if summer sprays were deferred until all adults had emerged, and hatching was approximately 50% complete.

Red pine (*Pinus resinosa* Ait.) and Scotch pine (*Pinus sylvestris* L.) are most widely planted in reforestation and tree farming operations in Michigan, and are attacked by the shoot moth more heavily than other pine species. Attacks on red pine, in particular, create a serious forestry problem and land management agencies have shown great interest in the use of insecticides for shoot moth control.

Haynes & Butcher (1961) have investigated the ecology of European pine shoot moth larvae and the relationship of this insect to *Pinus* spp. in Michigan. These and similar studies may ultimately suggest management practices which will make direct control unnecessary. In the meantime, foresters and tree farmers are concerned about existing stands now under attack by this pest.

Christmas tree growers and nursery men can obtain satisfactory control of shoot moth on all pine species with combinations of drench sprays and routine shearing (shaping) practices. If insecticides are to be acceptable in forestry practice, it is essential that they (1) have a low mammalian toxicity, and, (2) provide satisfactory control at safe, economical dosage levels. These two considerations dictated the course of the study reported here. The usefulness of these insecticides in low gallonage application equipment is also under investigation and will be reported elsewhere.

METHODS.—Applications of five insecticides which most nearly met the foregoing requirements were made to red pine April 23 and July 7 of 1960 at Mesick, Michigan. Each material was applied at 100 pounds pressure with a John Bean Spartan Sprayer at dosages of 2.0, 1.0 and 0.5 pounds actual toxicant per 100 gallons of water. Formulations used were: dimethoate 4 lb. EC (emulsion concentrate), DDT 2 lb. EC, methoxychlor 2 lb. EC, Sevin 85% sprayable, American Cyanamid SF60 (malathion plus sticker) 2.5 lb. EC, Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate 4 lb. EC and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate) 1.5 lb. EC. Design of the experiment was as follows:

Time of Application	*Size of Trees*	*Trees per Plot*	*Replications*
April 23	2–3 ft.	20	5
July 7	4–6 ft.	5	5

All treatments were randomized within each block.

At the time of application on April 23 larval feeding was well under way while on July 7 more than 95% of the adults had emerged and 30% of the eggs had hatched. On the basis of 1959 studies on shoot moth biology in relation to insecticide effectiveness, the April 23 spray timing was

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[2] The cooperation of the American Cyanamid Company, Pennsalt Chemicals Corporation, Geigy Chemical Corporation, Shell Chemical Corporation, and Chemagro Chemical Corporation is acknowledged; also the assistance of A. Wuotila, Vo-Ag Instructor, Buckley, Michigan in securing experimental plots.

Table 1.—Results of spring treatments in European pine shoot moth control.

Toxicant	Toxicant per 100 Gal. (lb.)	Mean No. Insects per Plot	Reduction from Check (%)[a]
Dimethoate	2.0	0.6	98.0
Dimethoate	1.0	1.0	96.6
DDT	2.0	1.2	95.9
Dimethoate	0.5	1.6	94.5
DDT	1.0	2.6	91.1
Methoxychlor	2.0	4.0	86.3
Sevin	2.0	6.0	79.4
Methoxychlor	1.0	6.2	78.7
Methoxychlor	0.5	6.2	78.7
DDT	0.5	7.0	75.9
Sevin	1.0	13.8	52.5
Sevin	0.5	15.0	48.3
SF60	2.0	15.0	48.3
SF60	1.0	16.6	42.8
SF60	0.5	22.0	24.2
Check	—	29.0	—

[a] Treatments with vertical line in common do not differ significantly from each other at the 5% level of significance using multiple range test (Duncan 1955).

very nearly optimum, while the July 7 application could have been deferred 3 or 4 days.

Table 1 ranks the spring treatments in terms of per cent reduction over the respective check. The figure "mean number of insects per plot" was obtained by dissecting out all larvae and pupae on each of the 20 trees in each plot on June 9 and 10, 1960.

Table 2 ranks the summer treatments in a similar way. Summer data on control were obtained by multiplying the number of infested shoots in a 250-shoot sample (50 shoots per tree) by the mean number of insects per shoot (based on laboratory dissection of five infested 2-inch shoot tips per tree). The statistics thus obtained in tables 1 and 2 were subjected to analyses of variance, and maximum significant differences were calculated using the significance table of Duncan (1955). Bayer 29493, Guthion, Am. Cyanamid Anti-feeding Compound 24055 (4′-(dimethyltriazeno) acetanilide) and a replicated shearing are included in the summer treatment. They are reported here because they appeared in the field experiment and represent additional degrees of freedom in the analysis. The effectiveness of Guthion confirms previous results. The results obtained with Bayer 29493, Am. Cyanamid Anti-feeding Compound, SF60 and shearing are of a preliminary nature.

Table 2.—Results of summer treatments in European pine shoot moth control.

Toxicant	Toxicant per 100 Gal. (lb.)	Mean No. Insects per Shoot × No. Infested in 250-Shoot Sample	Reduction from Check (%)[a]
Sevin	2.0	5.21	93.0
Dimethoate	1.0	6.54	91.2
Dimethoate	2.0	7.22	90.4
Bayer 29493	2.0	9.58	87.2
Guthion	0.5	9.65	87.1
Dimethoate	0.5	11.34	84.8
Sevin	1.0	17.40	76.8
Methoxychlor	2.0	17.66	76.7
DDT	2.0	20.39	72.7
SF60	1.0	24.74	66.8
Methoxychlor	1.0	25.86	65.3
SF60	2.0	32.73	56.1
Sevin	0.5	34.51	53.7
DDT	1.0	39.58	46.9
DDT	0.5	39.62	46.9
SF60	0.5	40.65	45.5
Methoxychlor	0.5	46.29	37.9
Shear		47.80	35.8
AFCpd	4.0	56.66	24.0
Check	—	74.56	—

[a] Treatments with vertical line in common do not differ significantly from each other at the 5% level of significance using multiple range test (Duncan 1955).

Figure 1 shows the graphed data on dimethoate, DDT, Sevin, methoxychlor and Am. Cyanamid SF60. Each insecticide is arranged on the abscissa in order of increased dosage. The control achieved with each insecticide in spring applications is shown as a curve while the summer application data are depicted as vertical bars. Both may be read as per cent control on the ordinate.

RESULTS.—As has been demonstrated in previous work (Butcher & Haynes 1959, 1960) dimethoate at 2.0 pounds gives excellent control of European pine shoot moth in both spring and summer applications. The performance of this insecticide at levels as low as 0.5 lb. in 100 gallons of water in this study suggests that still lower dosage may give satisfactory control when proper attention is given to timing. DDT performed well at the 1- and 2-lb. dosages (spring), but appeared to be less effective at 0.5 lb., indicating that this is very close to the minimum effective level. In summer applications even the 2-lb. dosage appeared to be inferior to dimethoate at 0.5 and Sevin at 1 lb., and not greatly different from methoxychlor or malathion at 1 and 2 lb. Methoxychlor compared favorably with DDT in spring treatments and showed the same decrease in effectiveness in summer treatments. None of the SF60 sprays differed significantly from the check in spring treatments, although both the 2- and 1-lb. applications showed such differences in the summer treatments. Bayer 29493 at 2 lb., and Guthion at 0.5 lb. gave good control of shoot moth in summer treatments. Neither the July 7 shearing nor the American

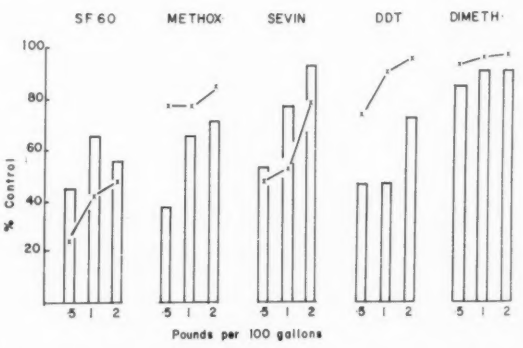

FIG. 1.—Per cent reduction in shoot moth population with spring (line) and summer (bar) applications of selected insecticides at three dosage levels.

Cyanamid Anti-feeding Compound at the 4 lb.-dosage reduced the populations significantly.

DISCUSSION.—DDT and dimethoate have proved useful in control of European pine shoot moth at dosages of 2 and 1 lb. per 100 gallons of water in hydraulic equipment. In the studies reported here, dimethoate gave excellent control in both spring and summer treatments at dosages as low as 0.5 lb. in 100 gallons. There was a sharp dropping off of DDT effectiveness in spring treatments as the dosage was reduced to 0.5 lb. in 100 gallons, and in summer applications neither the 0.5 nor the 1.0 levels gave significant reductions in population. Methoxychlor at similar dosage levels performed much like DDT, giving good spring control. At all dosages DDT and methoxychlor summer applications were inferior to corresponding dosages of dimethoate and Sevin.

Dimethoate appears unique (among compounds tested) in that the difference between the performance of comparable dosages in spring and summer applications was virtually negligible. Both DDT and methoxychlor gave inferior summer results when these were compared with their spring performance.

These results, in effect, corroborate earlier observations concerning the reduced effectiveness of DDT when applied in late June or early July against this insect, and conceivably can be interpreted as supplementing the already large body of evidence which attributes a negative temperature coefficient to this insecticide (Kearns 1956). The findings that methoxychlor did not differ materially from DDT in either spring or summer performance might commend its use where concern over possible undesirable side effects can negate the DDT cost advantage.

The investigations described here were suggested (in part) by results obtained in 1959 (Butcher & Haynes 1960) in which dimethoate and Sevin appeared to perform better than DDT when applied at the time approximately 50% of the eggs had hatched (summer). While DDT has been used extensively in both spring and summer treatments by Michigan growers, the evidence presented here strongly suggests that dimethoate (if commercially available) or Sevin would give superior control in summer applications.

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Artificial Nesting Sites for the Propagation of the Leaf-Cutter Bee, *Megachile* (*Eutricharaea*) *rotundata*, for Alfalfa Pollination[1,2]

W. P. STEPHEN, *Department of Entomology, Oregon State University, Corvallis*

ABSTRACT

The leaf-cutter bee, *Megachile rotundata* (Fabr.), although endemic to Eurasia, is found throughout most of continental United States where it has been reported polylectic on a variety of floral hosts. In the Pacific Northwest it is oligolectic on alfalfa, taking both pollen and leaf-cuttings from that plant. The bee does not excavate its own holes, but rather accepts any burrow or partially protected excavation that approximates its size.

The paper reports that the bee accepted holes bored in timbers and milk straws set in tin containers without any apparent discrimination. Such stocked containers are readily amenable to manipulation and storage. This flexibility would permit the grower to relocate stocked timbers and straws throughout the field each spring, thus insuring adequate pollination of his crop by this bee species.

During the past 10 years there has been mounting interest in the propagation of efficient wild bee pollinators of alfalfa for the purpose of increasing seed production. The author suggests that any given bee species must exhibit most of the following characteristics to be considered efficient: it must be oligolectic[3] on alfalfa; the species must be gregarious so that it can be placed in specific areas and populations there maintained; it must be a species that can be induced to nest readily in man-made or man-modified sites; it must be a species in which parasites and disease are not a problem or lend themselves to some method of control; it should be a species with a high reproductive potential so that gathering of large quantities of pollen is assured; the bee and the nesting site location should be amenable to manipulation so that the time of activity can be controlled; and it should have more than one generation per year to insure a rapid build-up of populations.

There have been several attempts to induce species of *Megachile* that are oligolectic on alfalfa to nest in holes bored in logs and in trap-nests composed of pithy-stemmed plants such as sumac and sunflower, but these have met with only limited success (Peck & Bolton 1946, Stephen 1955, Medler & Koerber 1958). This is attributable in part to the solitary habits, diverse nesting sites,

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[2] Dr. T. B. Mitchell of North Carolina State College suggests that there may be some question as to the validity in the application of the name *rotundata* to this species. This, however, can only be resolved through a revision of the subgenus.

[3] The term is discussed more fully in Stephen and Torchio (1961).

and the apparent preference of many leaf-cutter species to excavate their own burrows rather than accept existing tunnels.

The leaf-cutter bee, *Megachile* (*Eutricharaea*) *rotundata* (Fabr.), on the other hand, satisfies most of the above criteria. It has, however, only one generation per year, but the extended life span of the individuals throughout most, if not all, of the alfalfa-blooming period partially compensates for this.

Megachile rotundata is endemic to, and widespread in, Eurasia. It is believed to have been introduced into North America one or more times since the mid-1930's. The American and Eurasian host records for this species indicate that it is polylectic, taking its pollen from a wide variety of floral sources (Daly 1952, Hurd 1954, Osychiuk 1959, Ponomareva 1960). In the Pacific Northwest, however, the species is oligolectic on alfalfa (Stephen & Torchio 1961).

Published accounts indicate that there are many species of *Megachile* that are oligolectic on alfalfa throughout their range. However, there appear to be differences of opinion among research workers as to the oligolecty of other species on this crop. For example, *M. brevis* Say has been reported oligolectic on alfalfa in California (Linsley 1946) and in Utah (Bohart 1957), while in Manitoba it has rarely been observed taking pollen from this plant species (Stephen 1955). Similarly, *M. frigida* Smith is considered to be the principal pollinator of alfalfa in Manitoba (Stephen 1955) and Saskatchewan (Peck & Bolton 1946), while Hobbs & Lilly (1954) report that this species was common in southern Alberta, but rarely found on alfalfa. These reports of disjunct groups of populations of a given species reacting differently in their host specificity suggest a physiological polytypy. Although this phenomenon has been documented for only a few bee species, it can be expected in any genetically polymorphic species occupying a broad geographical range. *M. rotundata* appears to exhibit this same type of physiological polytypy, for this is the only area in which its oligolecty on alfalfa has been recorded. If its alfalfa specificity is genetically based, the selection of similarly responding phenotypes should be possible through some, if not all, of its range.

BIOLOGY.—*M. rotundata* is a small bee, 7 to 9 millimeters long. It has never been observed to construct its own burrow, but rather occupies existing holes that closely approximate its size. The cells are constructed in linear order as in other *Megachile*, with the total number of cells per nesting tube a function of the length of the tube that the particular female occupies. The cup of the cell is formed of a single or multiple layer of leaves into which the pollen mass is placed.

Females were not observed collecting pollen from any other competing plant although volunteer sweet clover, sunflower, Russian thistle, and mallow grew in the proximity of the nesting sites. The bees emerged during the first and second weeks of June, and were active until early October, at which time 10% of the population was still in flight. All of the leaf-cuttings used in the construction of the cell cup and the cell caps, as well as the plugs to the entrance of the tunnels, were composed of alfalfa leaflets or cuttings from alfalfa leaves. Their ability to utilize alfalfa as a source of both leaf cuttings and forage is believed to contribute to the establishment of large localized populations. The bee overwinters as a prepupa and there is only a single generation[4] per year in the areas in which these studies have been conducted (Stephen & Torchio 1961).

No parasites have been observed at the nesting site or reared from any of the overwintering prepupae during 1959 and 1960. If we assume that this species was introduced from Eurasia in recent times, then it is possible that the indigenous parasites in its area of endemism have not been introduced, or are lagging in their rapidity of dispersal.

These observations on *Megachile rotundata* by the author during 1958–60 in Eastern Oregon and Western Idaho have prompted investigations on increasing its numbers in the proximity of alfalfa fields through the development of artificial nesting sites.

METHODS.—The first localized population of *rotundata* was observed nesting in nail holes in an abandoned shack during the summer of 1958. There was a variation in the diameter of the holes accepted by the bees under natural conditions, but these diameters varied about a mean diameter of three-sixteenths of an inch. Approximately fifty three-sixteenths inch holes were drilled in the framing of the building in 1958, and all but a few of these were accepted and occupied during that season.

[4] Observations on *rotundata* were conducted in sheltered environs during the period reported on in this and a previous paper (Stephen & Torchio 1961). Since this manuscript was submitted, populations of this species have increased considerably and notes on artificial nesting sites exposed to direct sunlight during part of each day have been made. The straws or bored-beams, containing cells of this species and located in the direct sunlight for much of each day, yielded a second generation in the summer of 1961. A partial second generation was observed from artificial sites exposed to sunlight for only short periods during each day, and from those located in shaded areas where the temperatures were not markedly depressed by the shade. Many nesting sites situated in secluded areas, where the temperatures were reduced by 10 to 25 degrees caused by the shading, have yet to yield a second generation and undoubtedly will pass into the winter as first generation prepupae. The above-normal temperatures in June and July of this year (June average maximum temperature of 11 degrees F. above that recorded for June 1960 at Ontario, Oregon) contributed to the extensive second generation of the species. The second generation in *rotundata* appears to be controlled exclusively by the total amount of heat to which the cells are exposed. In 1961, emergence began in early June and by July 16 the first evidence of a second generation was noted from cells provisioned during the previous month. The emergence of a second generation has continued since that time, and apparently will continue until daytime temperatures decline.

The report by the author (Stephen & Torchio 1961) that the adults live for 3 to 4 months is obviously erroneous, and the observations on adult activity during early October were undoubtedly based on an inflight of second generation adults. The longevity of the adult in the Pacific Northwest ranges from 7 to 9 weeks.

FIG. 1a. Nests of *Megachile rotundata* in drilled holes in 4″ by 4″ timber.

FIG. 1b. Close-up of the timber showing the bee acceptance and the tunnel-end plugs of cut leaves.

FIG. 2a. View of prepared nesting sites *in situ*, showing three gallon cans above, and the "routed" lumber below.

FIG. 2b. Closeup of bee-occupied "routed" lumber. The cracks down the middle of each row of cells indicate the position of the glued faces.

FIG. 2c. Closeup of the entrances to the straws that were bulk packed into a gallon can.

FIG. 2d. Surface of a gallon can in which the straws were spaced separately but randomly. Darkened plaster of Paris fills the intervening areas to provide stability to the ends of the straws.

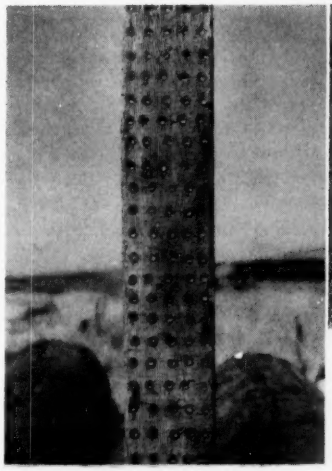


Fig. 1 a

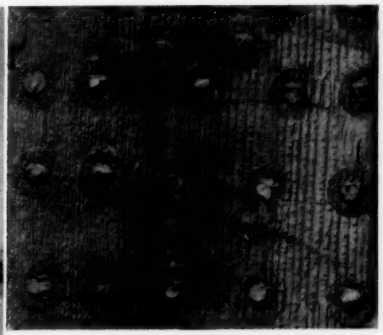


Fig. 1 b

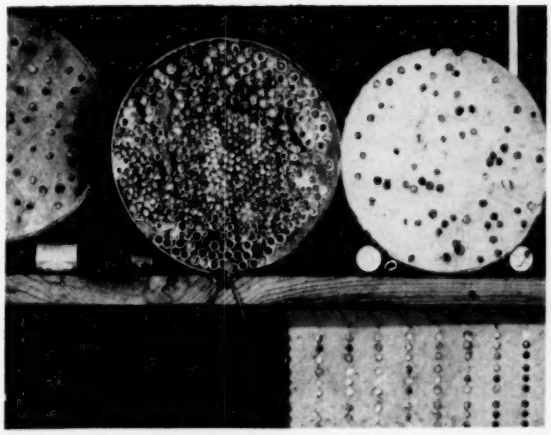
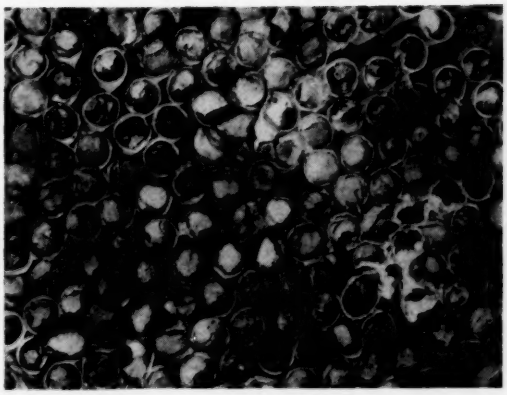


Fig. 2 a



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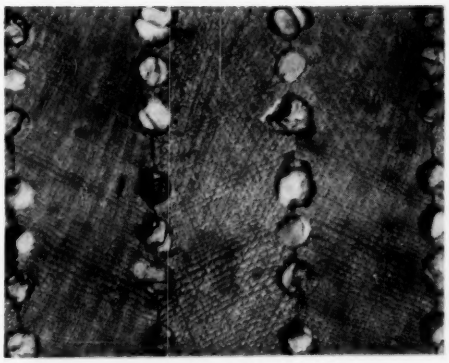

Fig. 2 b

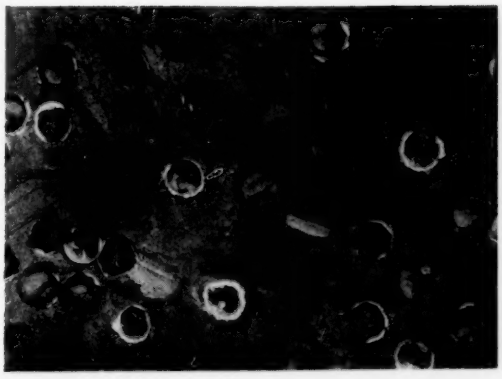

Fig. 2 d

In 1959, several hundred more holes were drilled in the frame of the building and during the latter part of the season, a 4″ by 4″ beam was perforated with holes of a similar diameter (figs. 1a, 1b). In 1960, additional drilled timbers were placed in the proximity of the nesting bees.

To ease the chore of drilling numerous holes in unwieldy wooden beams, a piece of lumber 6 inches wide by 6 feet long by three-fourths inch thick was grooved with a three-toothed router attached to a circular table saw. Each of the router-teeth was approximately three-sixteenths of an inch in diameter and three thirty-seconds inch deep. Thus, by passing both sides of the board through its full length over the router there remained half-holes on either side. The board was then cut in 4-inch lengths and folded together so that the semicircular grooves matched each other, forming near-perfect holes. The pieces were glued together and a sheet of thin plywood nailed over the back to seal the base (fig. 2a, 2b).

Because of the restricted depth to which holes could be drilled in 4″ by 4″ beams, "milk straws" with a diameter of three-sixteenths of an inch and a length of 6½ inches were inserted into gallon tin cans to determine their acceptability by the bees. The bottom of the can was filled to a depth of 1 inch with plaster of Paris into which the straws were inserted. The plaster of Paris was provided to offer stability to the straws, so that they would not, and could not, be removed readily. Vermiculite rather than plaster of Paris was then packed about the straws as a light weight filler. The top inch was then filled with plaster of Paris, darkened with a charcoal derivative to make the ends of the straws more readily visible and to darken their interior. In the first series of cans, the straws were spaced irregularly, but separately, so that the bees could more readily locate the straws selected as the nesting tube (figs. 2a, 2d).

A third can was prepared offering the bees both "milk straws" and "jumbo milkshake straws" of fifteen-sixty-fourths inch diameter, to determine whether there was any difference in the bee acceptability between the straws of the two diameters. The straws were packed closely and tightly together in the middle of the can, and randomly spaced near the margins (figs. 2a, 2c). It was planned to determine whether the mass of available nesting tubes would affect the ability of the nesting females to locate their tunnels.

RESULTS AND DISCUSSION.—The holes bored in the 4″ by 4″ beams, stationed immediately adjacent to the nesting area, were the first to be accepted by the emerging females. Once emergence had been completed from an overwintering tunnel, one of the later emerging females, presumably the last, cleaned the burrow and reoccupied it. The cells in the holes that were occupied earlier in the season terminated about an inch below the tunnel plugs.

The bees tended to act in a gregarious manner, filling all of the available holes in the proximity of the area of emergence before extending their activity to the adjacent sites. They failed to occupy any of the holes that extended through the timber or any of the holes in which day-light was visible at the terminal end.

All of the artificial domiciles were readily accepted by the bees, but as indicated in a previous paper (Stephen & Torchio 1961), they exhibited distinct preferences for nesting tubes of approximately three-sixteenths of an inch in diameter.

The can containing the straws spaced at irregular intervals was located closest to the area from which the bees emerged and was subsequently the first can to be utilized by the bees. After they had filled this can, they moved immediately to the second can containing the two sizes of straws in which the distance between the straws was determined by the irregularities associated with bulk packing (fig. 2c). The close proximity of the straws did not appear to retard the activity or to cause the females to spend undue amounts of time in locating their own nesting burrow. For the most part, each female would spend from 2 to 5 seconds flying over the surface of the straws and then dart quickly into her own tunnel.

Several of the "jumbo straws" were occupied by females and sealed with an end plug of from 7 to 30 cut-leaves. Examination of the contents of these larger straws revealed that only 2 of the 20 had completed cells located near the bottom. The others contained leaf cuttings which were irregularly packed into the bottom of the tunnel in an apparent attempt to reduce the aperture size for cell construction. All of these were abandoned and apparently plugged by the female.

The entire length of each of the nesting tunnels was utilized for cell construction in early- and mid-summer. However, as the season progressed, the behavior of the females became more erratic, and reproduction apparently became progressively less efficient. Many of the straws occupied during mid- and late-September had only one or two cells at their base. Others contained a series of from six to 12 cells, complete with pollen, cell caps and end plugs, in which the eggs were all nonviable. The straws occupied during mid-summer were usually filled to capacity with from 10 to 14 cells. The holes drilled into the 4″ by 4″ timbers contained from three to six cells with an average of five cells in the 14 nesting tubes examined. The efficiency in utilizing the bees for pollination purposes would thus appear to be greater in the case of the longer nesting tubes where the number of tunnel-end plugs is reduced.

Each female completed more than one nesting tube in the course of her life, but the number of cells constructed by each female is as yet unknown. The preliminary data accumulated during the past 3 years suggests that each female may construct well in excess of 30 cells a year.

Unlike many other *Megachile*, *rotundata* remains active in a very restricted area when sufficient nesting sites are available to it. Females have been noted to return to the nesting tunnel with leaf-cuttings in 10 to 20 seconds, and with pollen loads in 1½ to 3 minutes after their departure. This would suggest that they obtain both in close proximity to the nesting site. These observations, as well as those made on its activity in the field, indicate that even large populations limit their activity to an area less than one-quarter of a mile in diameter about the nesting site.

The bees accepted the holes bored in the timbers, the laminated boards, and the "milk straws" without any apparent preference for either. The straws, however, were longer, accommodating more cells than the holes bored in the 4″ by 4″ timbers, and the efficiency of each

female was improved in the case of the straws by eliminating the need for frequent tunnel-end plugs in the shorter tunnels.

The timbers, and, more particularly, the containers filled with straws are readily amenable to manipulation and storage. This flexibility would permit the grower to relocate stocked timbers or straws throughout the field each spring, thus insuring adequate pollination of his crop by this bee species.

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Systemic and Contact Insecticidal Effectiveness of Selected Chemicals Administered Orally or Dermally to Rabbits[1]

H. W. DOROUGH and B. W. ARTHUR, *Department of Zoology-Entomology, Auburn University Agricultural Experiment Station, Auburn, Alabama*

ABSTRACT

Butonate, Monsanto CP 10502 (1-(dimethoxyphosphinyl)vinyl dimethyl phosphate), phosphamidon, and Sevin® (1-naphthyl *N*-methylcarbamate) administered as a single oral dose to rabbits were quite effective against the bed bug (*Cimex lectularis* L.) taking a blood meal, but were less effective against the Gulf Coast tick (*Amblyomma maculatum* Koch). Dimethoate and Shell SD 3562 (2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate) applied dermally to rabbits at 100 mg. per kg. were highly effective as systemics against the bed bug for 5 and 48 hours posttreatment, respectively; Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) was slightly less effective. As a contact insecticide, the residual effectiveness of Sevin was considerably longer than several other compounds tested.

Previous reports on the evaluation of a potential animal systemic insecticide involved a single oral administration of the chemical to laboratory animals and recording the mortality of various ectoparasites taking a blood meal from the treated host (Adkins et al. 1955, Drummond 1958, Flynn & Eden 1958, Brady et al. 1960, Flynn & Eden 1960). Subcutaneous (Drummond 1958, Hewitt et al. 1958) and intramuscular (Hewitt et al. 1958, Vickery & Arthur 1960) treatments of the host have been included in some investigations.

Dermal treatment of laboratory animals has not been reported as a routine screening procedure. The effectiveness of Co-Ral®(Bayer 21/199) when applied dermally (Brundrett et al. 1957) as compared with its ineffectiveness when applied orally (Adkins et al. 1955) emphasizes the importance of including dermal treatments in initial screening programs. Other materials, such as Dowco 109 (O-4-*tert*-butyl-2-chlorophenyl) O-methyl methylphosphoramidothioate) (Roth & Eddy 1959) and Ruelene® (O-4-*tert*-butyl-2-chlorophenyl O-methyl methylphosphoramidate) (Rogoff & Kohler 1960, Kohler & Rogoff 1961), were effective animal systemic insecticides when administered orally or dermally.

The effectiveness of several insecticides applied orally or dermally to rabbits for control of certain bloodsucking ectoparasites is reported in this paper.

PROCEDURE.—*Materials.*—The technical materials used in this study included:

Bayer 23453, *O,O*-dimethyl *S*-(2-ethylethyl)sulfinyl phosphorodithioate

Bayer 24498, *O,O*-dimethyl *S*-(methylsulfinyl)ethyl phosphorothioate

Bayer 25198, *O,O*-dimethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate

Bayer 23655, *O,O*-dimethyl *S*-(2-isopropyl)-ethylsulfinyl phosphorothioate

Bayer 29493, *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate

Bayer 30911, methyl-*O*-methyl *O*-2,4-dichlorohpenyl phosphonothioate

[1] This investigation was supported in part by a research grant PHS E-549 from the Division of Research Grants of the National Institutes of Health, Public Health Service. Original manuscript forwarded July 28, 1960, became lost en route. This copy accepted for publication May 15, 1961.

Table 1.—Systemic effectiveness of several compounds against bed bugs and ticks when administered as a single oral dose to rabbits.

Compound	No. Rabbits Treated	Dose to Rabbit (mg./kg.)	Per Cent Mortality[a]	
			Bed Bugs	Ticks
Butonate	3	100	96 (50)	32 (38)
Bayer 23655	2	50	100 (34)	100 (9)
	2	25	100 (44)	47 (19)
Bayer 30911	2	10	0 (44)	—[b]
Dimetilan	2	50	0 (41)	—[b]
	2	25	0 (39)	15 (52)
Dowco 109	4	100	0 (45)	1 (80)
Geigy G-30494	2	100	0 (56)	25 (36)
Gen. Chem. 3582	2	50	0 (43)	0 (39)
Gen. Chem. 3583	2	50	0 (47)	0 (51)
Gen. Chem. 4072	3	50	0 (39)	0 (40)
Methyl Trithion	3	100	0 (46)	0 (43)
Monsanto CP 10502	3	50	100 (72)	100 (62)
	4	25	100 (91)	74 (23)
	5	10	71 (104)	22 (23)
	2	5	0 (36)	0 (43)
Phosphamidon	2	10	100 (42)	65 (37)
Ruelene	4	100	0 (86)	17 (79)
	2	50	0 (52)	0 (44)
Sevin	3	100	66 (65)	0 (49)
Shell SD 5539	2	10	0 (41)	22 (18)
VC 1-13	2	100	0 (48)	0 (36)

[a] Number of bed bugs and ticks feeding on rabbits are shown in parentheses.
[b] Rabbits died before mortality counts were made.

Butonate, *O,O*-dimethyl 2,2,2-trichloro-1-*n*-butyryloxyethyl phosphonate
Dimetilan®, 3-methyl-5-pyraxolyl dimethylcarbamate
Dimethoate, *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
Dowco 109, O-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidothioate
Geigy G-30494, *O,O*-dimethyl *S*-2,5-dichlorophenylmercaptomethyl phosphorodithioate
Gen. Chem. 3582, *O,O*-diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate
Gen. Chem. 3583, *O,O*-diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate
Gen. Chem. 4072, *O,O*-diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate
Methyl Trithion®, *O,O*-dimethyl *S*-[(*p*-chlorophenylthio)methyl]phosphorodithioate
Monsanto CP 10502, 1-(dimethoxyphosphinyl)vinyl dimethyl phosphate
Phosphamidon, 1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate
Ruelene®, *O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate
Sevin®, 1-naphthyl *N*-methylcarbamate
Shell SD 3562, 2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate
Shell SD 5539, *m*-nitrobenzyl 3-(dimethoxyphosphinyloxy) crotonate
VC 1-13, *O,O*-diethyl *O*-2,4-dichlorophenyl phosphorothioate

Oral Treatments.—Rabbits weighing from 4 to 5 kg. were treated with a single oral dose of the compounds listed in table 1. The compounds were dissolved in corn oil and administered through a Bardam No. 12 Fr. urinary catheter; from two to five rabbits were treated with each material. Two days prior to treatment about 25 nymphs of the Gulf Coast tick (*Amblyomma maculatum* Koch) were placed in feeding chambers attached to a shaved area on the back of the rabbit and at 1 hour after treatment about 25 fifth-instar bed bugs (*Cimex lectularis* L.) were allowed to take a blood meal from the treated host (Adkins et al. 1955). The effectiveness of each material was based on the percentage mortality of ticks that died on the host and from the percentage bed bug mortality at 24 hours after completion of the blood meal.

Dermal Treatments.—The appropriate amount of each compound corresponding to the mg. per kg. dosage given in table 2 was dissolved in 2 ml. xylene and 1 ml. Triton X-100 and applied to the shaved back of a rabbit with a 5-cc. syringe without needle. A specially constructed yoke was placed around the neck of each rabbit to prevent licking or oral ingestion of the applied chemical. From two to four rabbits were treated with each material. Fifth-instar bed bugs and nymphs of the Gulf Coast tick were confined in a feeding chamber on an untreated portion of the back. Ticks were allowed to attach 2 days prior to treatment. For systemic evaluation, bed bugs were placed in the feeding chamber at 1, 5, 24, 72, and 96 hours after treatment. The bed bugs that refused to feed to repletion were held as controls and served as an indicator as to whether the feeding chamber was contaminated from the dermal application of insecticide. In some instances, bed bugs that had fed on an untreated animal were placed in the feeding chamber as controls. Results of the systemic effectiveness were expressed as percentage bed bug mortality 24 hours after feeding and as percentage tick mortality occurring on the host.

The contact effectiveness of the compound was evaluated concurrently with the systemic effectiveness (table 2). Bed bug nymphs were allowed to take a blood meal from the treated portion of the shaved back of the rabbit at the same time intervals used for systemic evaluations. Mortality of bed bugs may have resulted in part from ingestion of toxic blood or from contact with the insecticide on the skin of the rabbit.

RESULTS.—*Oral Treatment.*—Butonate, Monsanto CP 10502, phosphamidon, and Sevin when administered as a single oral dose to rabbits were quite effective against bed bugs taking a blood meal (table 1). Monsanto CP 10502 (50 mg. per kg.) was the only compound highly effective against the Gulf Coast tick, but the effectiveness of this material declined rapidly as the dosage was lowered to 25, 10, or 5 mg. per kg. Phosphamidon and Bayer 23655 were the next most promising materials against the tick.

All of the compounds (table 1) were not administered at sublethal dosages to the rabbits. Notes made on the toxicity of the compounds were as follows: Bayer 30911 at 100, 50, or 10 mg. per kg., all rabbits died; Dowco 109 at 100 mg. per kg., two out of four rabbits died; Gen. Chem. 4072 at 50 mg. per kg., one out of three rabbits died. Other compounds (table 1) were apparently nontoxic to the rabbit at the indicated dosage administered.

Dermal Treatment.—Dimethoate and Shell SD 3562 applied dermally to rabbits at 100 mg. per kg. were 100% effective as systemics against the bed bug for 5 and 48 hours posttreatment, respectively (table 2). Bayer 29493 was slightly less effective than Shell SD 3562 as a dermal systemic against bed bugs. There was no mortality of bed bugs held as controls on the treated animals, indicating that the feeding chambers were not contaminated with the dermal application of insecticide and that mortalities were due to ingestion of toxic blood. Bayer 29493 and

Table 2.—Contact and systemic activity of several compounds against bed bugs and ticks when administered dermally to rabbits.

Compound	No. of Rabbits Treated	Dose to Rabbit (mg./kg.)		Per Cent Mortality at Indicated Hours						
				Bed Bug						
				1	5	24	48	72	96	Tick
Bayer 23453	3	100	Systemic[a]	0	27	0	0	0	0	15
			Contact[b]	100	100	93	39	0	0	—
Bayer 24498	3	25	Systemic	33	34	53	0	0	—	35
			Contact	100	100	70	33	0	—	—
Bayer 25198	3	25	Systemic	69	68	0	0	0	—	26
			Contact	100	100	100	53	100	—	—
Bayer 29493	3	100	Systemic	86	89	93	100	59	68	92
			Contact	100	100	100	100	85	46	—
Dimethoate	2	100	Systemic	100	100	56	0	0	—	93
			Contact	100	100	100	60	0	—	—
	3	50	Systemic	95	26	0	0	0	—	0
			Contact	100	100	73	36	0	0	—
Monsanto 10502	2	100	Systemic	100	100	0	0	—	—	63
			Contact	100	100	100	80	92	—	—
Sevin[c]	4	100	Systemic	13	0	0	0	0	—	0
			Contact	100	100	100	100	100	100	—
Shell SD 3562	2	100	Systemic	100	100	100	100	71	75	57
			Contact	100	100	100	100	100	100	—
	3	50	Systemic	100	58	57	0	—	—	41
			Contact	100	100	100	7	—	—	—

[a] Bed bugs and ticks confined to feeding chambers on untreated portion of back to evaluate systemic effect.
[b] Bed bugs exposed to treated area of back at indicated time intervals.
[c] Sevin was also effective (contact) against bed bugs as follows: 120 hours (100%); 144 (100%); 168 hours (100%); 192 (89%); 216 (52%); 240 (56%).

dimethoate were the most effective materials against ticks (systemic evaluation).

As a contact insecticide, the residual effectiveness of Sevin was considerably greater than that of other compounds (table 2, footnote c). Sevin was 100% effective (contact) against bed bugs for 7 days after treatment of rabbits; Shell SD 3562, 3 days; Bayer 29493, 2 days; Bayer 25198, 1 day; dimethoate, 1 day; and Monsanto CP 10502, 1 day.

Bayer 24498 and Bayer 25198 were more toxic when applied dermally to rabbits than other compounds shown in table 2. One out of two rabbits died when treated with Shell SD 3562 at 100 mg. per kg.

Discussion.—Dermal applications of insecticides to host animals should be included in screening programs for determining the systemic effectiveness of candidate materials. Some materials are effective against cattle grubs when administered orally (Adkins 1957); others are effective when applied dermally (Brundrett et al. 1957); yet others are effective by either route of administration (Roth & Eddy 1959, Rogoff & Kohler 1960, Kohler & Rogoff 1961). The systemic activity of Co-Ral might have been discovered earlier had dermal applications been included in initial work.

The lack of systemic activity of Dowco 109 and Ruelene administered orally to rabbits (table 1) was not anticipated, since these two materials are quite effective against cattle grubs (Roth & Eddy 1959, Rogoff & Kohler 1960). These are the only two compounds tested in this laboratory possessing such a unique toxicity pattern. Bed bugs placed on Dowco 109-treated rabbits at 2- to 4-hour intervals for 24 hours lived and molted normally. Thus, the 1-hour posttreatment interval could not be used to explain the lack of activity of this material. Apparently, rabbits are more efficient than cattle in degrading these materials to nontoxic products or there was a wide selectivity difference between insect species (cattle grub and bed bug). Usually, the bed bug is a very sensitive bioassay organism in evaluating animal systemic effectiveness, and it is more sensitive than the Gulf Coast tick (Brady et al. 1960). The stable fly may be even more sensitive than the bed bug to certain toxicants (Flynn & Eden 1960).

Techniques used in dermal applications were adequate to assess the systemic and contact toxicity of the compounds. Feeding chambers were not contaminated with the insecticides, and the yokes around the neck of the rabbits prevented oral ingestion of the applied material.

The systemic effectiveness of compounds containing alkylcarbamoyl groups (dimethoate, phosphamidon, and Shell SD 3562) and thioether groups (Bayer 23453, Bayer 23655, Bayer 24498, Bayer 25198, and Bayer 29493) was anticipated based on results of previous studies (Brady et al. 1960). Butonate is a phosphonate (Arthur & Casida 1958) structurally similar to Dipterex® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate) that has animal systemic activity (McGregor et al. 1954). Bayer 30911, a 2,4-dichlorophenyl phosphonothioate, was inactive as an animal systemic, but the activity of certain 2,4-dichlorophenyl phosphorothioates has been reported recently (Flynn & Eden 1960).

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The Use of Balsam Fir Shoot Elongation for Timing Spruce Budworm Aerial Spray Programs in the Lake States[1]

J. L. BEAN, *Lake States Forest Experiment Station, Forest Service, U. S. Department of Agriculture, St. Paul, Minnesota*

ABSTRACT

Studies in Minnesota in spruce-fir stands heavily depleted by the spruce budworm (*Choristoneura fumiferana* (Clemens)) have shown that larval development was closely correlated with balsam fir shoot elongation. With this as a basis, a method was developed using balsam fir shoot elongation for timing spruce budworm aerial spray programs in the Lake States. Two methods of calculating total shoot growth for the coming season are presented in this paper.

Since 1954 the current outbreak of the spruce budworm (*Choristoneura fumiferana* (Clemens)) in northeastern Minnesota has increased in area and intensity throughout the spruce-fir stands. Top killing and tree mortality is apparent on about 96,000 acres along the Canadian border. Extensive control programs, consisting of aerial application of DDT sprays, to prevent future damage are being considered by timberland owners. The success of these control programs depends, among other things, on the timing of the spray application which must coincide with that period in larval development when a maximum amount of foliage protection, as well as an immediate reduction of the budworm population, can be obtained.

Webb (1955), working in New Brunswick, noted that a significant amount of foliage was protected when sprayed at the peak of the third larval instar or when the buds had begun to open and expose the new needles. Furthermore, the effectiveness of the spray increased to a maximum 10 days after the peak of the third instar and then declined rapidly.

Morris et al. (1956) found that shoot elongation of balsam fir could be used as a simple and objective method for timing insect collections over a large forested area, and that it would be of particular importance in the timing of aerial forest spraying programs. They also noted that a knowledge of phenological differences over such an area would be of value in planning the logistics of extensive control operations and in determining the sequence in which different parts of the area should be treated.

According to Greenbank (1956), the initiation of larval development could not always be closely synchronized with the initiation of shoot development. When emergence of the larvae from hibernation preceded shoot activity, second-instar larvae resorted to mining needles, vegetative buds, and staminate flowers. Later, as shoot elongation started, the larvae moved to the newly developing shoots. Although mining of needles and buds enabled the larvae to survive until shoot development occurred, it did not materially speed up larval development. However, Blais (1952) found that budworm larvae which fed on staminate flowers had an advantage of about 3 days in development over larvae feeding exclusively on foliage. Consequently, in flowering years, if emergence is especially early and shoot development late, the budworm larvae will have developed more relative to the shoots than in years when the two events are more closely synchronized.

Observations in northeastern Minnesota showed that little or no flowering took place in balsam fir stands that had suffered 2 or more years of heavy defoliation. Those trees that did flower were scattered throughout the stand and did not occur in significant numbers. Apparently, heavy defoliation by the budworm inhibits flower production. Therefore, in areas proposed for control, where heavy defoliation has occurred for 3 or more years, the

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development of the budworm is nearly synchronized with shoot development.

The study reported here was undertaken to determine if this relationship between larval development and shoot growth could be used for timing spray operations.

METHOD.—The procedure used in this study was based, in part, on those outlined by Morris et al. (1956). In cooperation with State, Federal, and private timberland managers 19 sampling stations were established throughout the spruce-fir type in northeastern Minnesota (fig. 1). At each of these stations 10 well-exposed, vigorous balsam fir trees about 20 feet tall were selected. On each tree one terminal shoot of a primary branch within easy reach was tagged for measuring. These shoots were measured to the nearest millimeter at 4- to 7-day intervals, starting before vegetative activity occurred. All measurements were started at the base of the bud (fig. 2).

A similar sampling station was established in the vicinity of the Kawishiwi Field Laboratory near Ely, Minn., with data taken in the same manner. Thus, when all data had been analyzed, the probable sequence of events at the various other sampling stations could be estimated from their time of occurrence at the Kawishiwi Station.

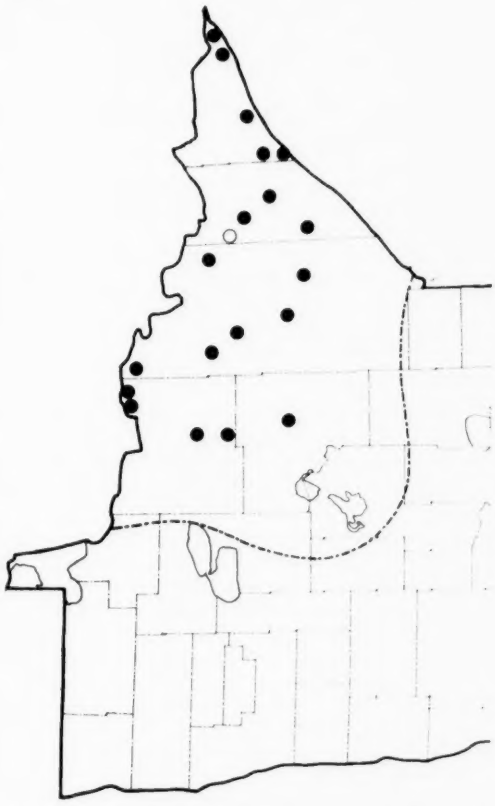

FIG. 1.—Map of northeastern Minnesota showing distribution of sampling stations. Broken line shows the approximate range of balsam fir in this section of Minnesota. Open circle denotes the station near the Kawishiwi Laboratory.

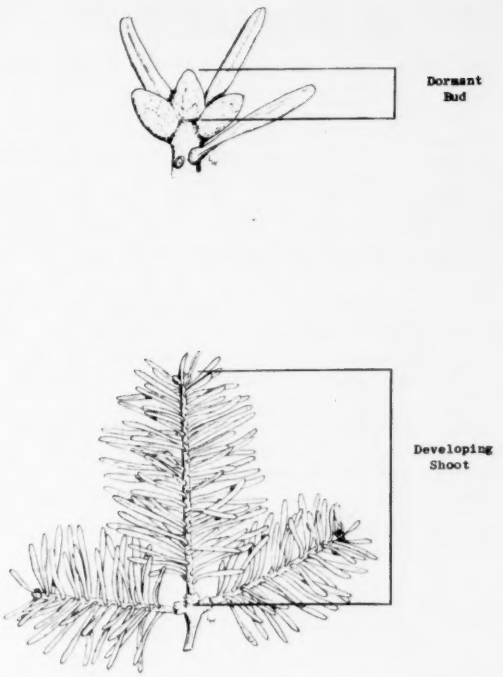

FIG. 2.—Method of measuring lateral growth of balsam fir.

In addition to shoot measurements, larval collections were made at regular intervals in the vicinity of four of these sampling stations, including Kawishiwi. The per cent of larvae in each instar was determined for each of these collections.

In the analysis of the data on shoot measurements, the initial length of the bud was first subtracted from each measurement. The remainder represented the cumulative shoot growth that had occurred during the season up to the date when the final measurement was made—usually after vegetative activity had ceased. For convenience in the final analysis, all growth measurements are expressed as a per cent of the total shoot growth for that season.

DISCUSSION.—When the shoot growth data at each sampling station were summarized and the mean growth plotted for several years, a typically sigmoid type of curve was produced. This curve varied somewhat between stations, particularly during the early part of the growing season. In general, growth ceased at all stations at approximately the same time.

The data on larval development were summarized and the date when the peak of the third instar was reached was determined. The per cent of total shoot growth that had taken place up to this date was calculated for each station where larval collections were made. These data are given in table 1 for each year that larval collections were made. When the data for all collections for all four stations were averaged, it was found that approximately 15% of the total annual shoot growth had taken place by the time larval development had reached the peak of the third instar in the field.

Table 1.—Dates when peak of third instar occurred at four separate locations, with per cent of total shoot growth for each date.

Plot Location	Date (May) Peak of Third Instar			Per Cent of Total Shoot Growth			Average	
	1957	1958	1959	1957	1958	1959	Date (May)	Per Cent Growth
Kawishiwi	24	26	25	10	18	14	25	14
Two Harbors		30	29		19	15	29	17
Crane Lake	25	21	23	16	15	12	23	14
International Falls		21	25		14	18	23	16

These results strongly indicated the possibility of predicting larval development by simply measuring per cent of total balsam fir shoot elongation. Of course, a method of estimating total shoot elongation had to be developed before the prediction system could be used.

At each of the four stations where larval collections were made, the annual shoot growth for 1955 through 1959, measured on 10 branches, was subjected to the following analysis:

1. The average internode length ($\bar{x}_i$) over the five growing seasons was calculated for each branch.

$$\bar{x}_i = \frac{\sum_{j=1}^{5} x_{ij}}{5} \quad (ij \text{ indicates the } i\text{th branch and the } j\text{th year})$$

2. The ratio (y_{ij}) of each year's internode length to the average internode length was found for each branch.

$$y_{ij} = \frac{x_{ij}}{\bar{x}_i}$$

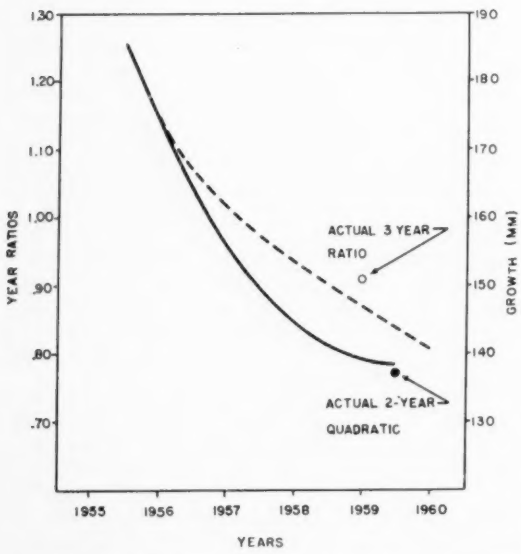

FIG. 3.—Onion River spray area. Shoot growth curves derived from 3-year ratios (dash line) and 2-year quadratic composite data (solid line). Ratios represent internode length related to average 5-year internode length.

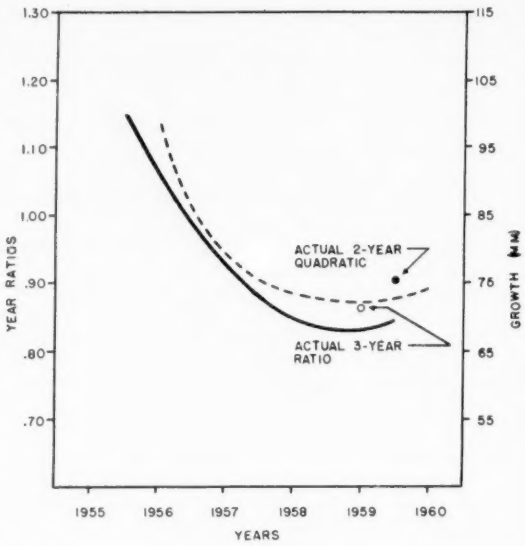

FIG. 4.—Meander Creek spray area. Shoot growth curves derived from 3-year ratios (dash line) and 2-year quadratic composite data (solid line). Ratios represent internode length related to average 5-year internode length.

3. The average of these ratios ($\bar{y}_j$) for the 10 branches was found for each year.

$$\bar{y}_j = \frac{\sum_{i=1}^{10} y_{ij}}{10}$$

4. A direct plot of these averages against time resulted in an irregular curve. Three-year averages (p) were used to smooth the curve.

$$p\,1956 = \frac{\sum_{j=1}^{3} \bar{y}_j}{3}, \qquad p\,1957 = \frac{\sum_{j=2}^{4} \bar{y}_j}{3}, \qquad p\,1958 = \frac{\sum_{j=3}^{5} \bar{y}_j}{3}$$

5. These p values were then plotted (figs. 3–5) and the curve was projected to give an estimated p value for 1960.
6. Finally, p times $\bar{x}$ for each branch gave the shoot growth expected in 1960 for that particular branch. The expected 15% shoot growth was calculated from this.

This method of fixing spray dates was field tested during the 1960 field season. Five separate areas involving a total of 23,000 acres had been set up for a spruce budworm control operation. Previous experience had shown that larval development would be slower in some of these areas than in others, but it was not known how much slower.

Three of these areas were selected for the study. The remaining two were close to one of the other three. The Kawishiwi Station was also included, as this station is a permanent one. In the vicinity of each of the three selected spray areas, a sampling station was established, and 10 vigorous young balsam fir trees were picked for sampling. Two branches on each tree were tagged for measuring—one on the north side and one on the south side of the tree. The annual shoot growth for the past 5

Table 2.—Comparison of dates (May) for peak of third-instar development and occurrence of 15% of shoot growth.[a]

Location	Peak of Third Instar	Date When 15% of Shoot Growth—	
		Was Expected	Actually Occurred
Meander Creek	22	23	25
Onion River	27	29	27
Pike Lake	26	28	25
Kawishiwi	23	24	24

[a] Spraying operation should be scheduled for 10 days after peak of third-instar development.

years (1955–59) was measured and the expected shoot growth for each branch for 1960 calculated by the method outlined above. The expected 15% growth for each branch was then calculated from these data.

In the spring of 1960, shoot growth on each branch was measured at regular intervals, these intervals becoming shorter as the 15% point was approached. In addition, larval collections were taken whenever shoot measurements were made, and the per cent of larvae in each instar determined.

The date when the peak of the third instar was reached at each sampling station, the date when 15% shoot growth was expected, and the date when it actually occurred all coincided very closely (table 2).

During the study it was observed that some shoots developed much faster than others, while some did not develop at all. If one waited until all growing shoots had reached the 15% point before making his calculations, the proper timing of the spray program would be seriously affected To overcome this problem, measurements were discontinued when 80% of the shoots had reached 15% of their expected growth. This made possible the elimination of those buds that did not develop and also those that exhibited more than the usual rate of growth. A review of the data showed that the 80% level provided a sufficient number of measurements to develop growth curves.

Although this method of determining expected shoot growth provides a useful "field" approach, it permits some variation in results because the curves are drawn freehand. If a mathematical expression for estimating a year's growth by relating the growth of previous years to tree age could be developed, the subjectivity of the above method would be eliminated.

With this in mind, the data from Onion River, Meander Creek, and Kawishiwi were subjected to some curve fitting. The best type of curve for estimating growth (y) for a year based on the relation between growth and age (x) for the preceding 5 years was found to be: $y=a+bx+cx^2$ (figs. 3–5). The criteria for judging each type of curve were (1) the average difference between the actual and estimated values for a new set of data, and (2) the standard error of this difference.

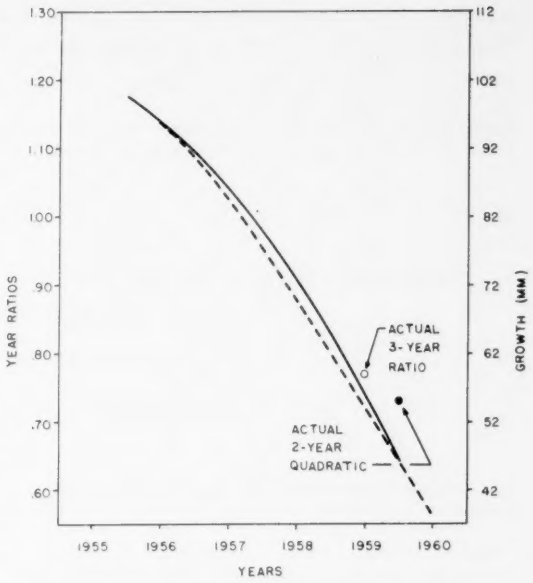

FIG. 5.—Kawishiwi Field Laboratory. Shoot growth curves derived from 3-year ratios (dash line) and 2-year quadratic composite data (solid line). Ratios represent internode length related to average 5-year internode length.

Fitting a separate curve to each branch did not give the necessary accuracy. It was only when the expression was based on all branches at a location that this accuracy was obtained. The final estimate for each branch was found by adjusting the estimate for each location by the relative rate of growth of the branch compared with all branches used at that location.

Only at Kawishiwi was there a significant difference between actual and estimated branch elongation for 1960. The standard error for all three stations was consistently low. The Kawishiwi data showed no significant difference when expressed by a linear equation; however, these data had a high standard error. Table 3 gives the parameters for the quadratic composite analysis for each area.

In making the above analysis the IBM 650 computer was used to obtain the various coefficients for the curves tried, which included the linear, quadratic, and cubic expressions for 49 branches separately and for the three areas.

Shoot growth measurements will be continued for at least 3 years to determine yearly variations between dates based on expected growth and those based on actual growth. The procedure, however, is presented at this time as a useful tool for dating spruce budworm aerial spray programs.

Table 3.—Summaries of the quadratic composite analysis in days for each area.

Area	Mean Difference (in days)	Standard Error of the Mean Difference	95% Confidence Interval (in days)
Kawishiwi	4.555	0.5087	4.555 ±1.175
Onion River	.5500	.2083	.5500 ± .4353
Meander Creek	1.421	.8762	1.421 ±1.840

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Dormant Sprays for the Control of the Pear Psylla, *Psylla pyricola*, in British Columbia[1]

HAROLD F. MADSEN,[2] *University of California, Berkeley*
and
JAMES MARSHALL, *Canada Department of Agriculture, Summerland, British Columbia*[3]

ABSTRACT

An experiment was undertaken to determine the value of dormant oil and dormant oil-insecticide mixtures for control of the pear psylla, *Psylla pyricola* Förster.

Delayed dormant application of dormant oil-lime sulfur mixture was as effective as a dormant application made 9 days earlier. The dormant oil-lime sulfur mixture was less effective than dormant oil alone. A dormant oil of 405 seconds Saybolt viscosity at 100° F. was more persistent and more effective than an oil of 205 seconds viscosity. Polybutene (Indopol H-100) provided initial control equal to the more viscous dormant oil, but reinfestation occurred within 3 weeks. Analysis showed the polybutene to be an extremely persistent compound. Dormant oil-dinitrocresol provided the best control. The mixture reduced the adult population to low numbers and prevented egg deposition on the twigs for 30 days. None of the spray mixtures were phytotoxic. The plots treated with dormant oil (205 vis.), experimental dormant oil (405 vis.), and dormant oil-dinitrocresol did not show psyllid numbers at treatment level until late July.

One of the first investigations on winter control of the pear psylla (*Psylla pyricola* Förster) was with the use of petroleum oils. Ross (1925, 1926) in Ontario, Canada, tested lubricating oils emulsified with Bordeaux and reported that pear psylla control was obtained. In advocating the use of an oil of 200 to 250 seconds Saybolt viscosity at 100° F., Ross (1932) emphasized that the primary effect of the oil was to prevent oviposition and not to kill the overwintering psyllid adults.

In 1942, shortly after the pear psylla had become established in the Pacific Northwest, a project was undertaken by collaborators in the Western Cooperative Spray Project to investigate two types of oils for pear psylla control. As supplied to the collaborators, one oil was the so-called standard dormant oil with an unsulfonated residue of 63% and a viscosity at 100° F. of 105 seconds Saybolt. The second oil had an unsulfonated residue of 66% and a viscosity of 250 seconds Saybolt. Both oils were of the same base. In British Columbia, pear psylla populations were low when the oils were received; therefore, no data were obtained on the effect of the oils on control of this insect. But, over a 5-year period, a considerable amount of work was done on the effects of these oils on other insects and on phytotoxicity (Marshall 1948). The more viscous dormant oil not only proved to be the better insecticide but also was less phytotoxic than the standard (105 seconds) dormant oil. The more viscous oil was recommended to the British Columbia fruit growers in 1945 and since that time has been universally used in the Province. In 1959, for the first time since the discovery of the pear psylla in British Columbia (Marshall & Olds 1947), the insect became a serious pest; and it was feasible to examine its susceptibility to dormant oils and oil-insecticide combinations. In addition, it was possible to ascertain if dormant or delayed dormant timing was best for control.

MATERIALS AND METHODS.—In the spring of 1960, experimental spray mixtures were applied on a 3-acre plot of 15-year-old Bartlett pear trees planted 20 feet apart. There were seven treated plots, with 26 to 49 trees per plot and one unsprayed block of 23 trees. The sprays were applied to single blocks of trees with the exception of the dormant oil-lime sulfur mixture. This latter combination was applied to two plots, one at each end of the experimental orchard.

The treatments were made with a concentrate sprayer (Okanagan Experimental Sprayer, built and designed by the Agricultural Engineering Section, Summerland Research Station) of the following specifications: single side, power take-off, air output of 7,000 cubic feet per minute at a velocity of 140 miles per hour, liquid output 3 gallons per minute at a pressure of 100 pounds per square inch.

The spray dosage varied from 61 to 91 gallons per acre, depending upon terrain and type of spray mixture. The machine was operated at a speed of approximately 1.75 miles per hour. Oil deposits were determined by chemical analysis 1 day after the spray was applied. The persistence of the deposits was obtained by repeating the determinations after the deposits had weathered for some time.

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[2] On sabbatical leave to Summerland, British Columbia, in 1960.

[3] The writers acknowledge with thanks the assistance of K. Williams and F. E. Brinton, chemists at the Entomology Laboratory of the Regional Research Station, Summerland, who carried out the oil deposit determinations that are summarized in table 1.

Table 1.—Dormant oil and polybutene deposits on pear twigs following application of dormant and delayed dormant spray mixtures. Summerland, B. C. 1960.

Plot	Material[a]	Gallons Applied per Acre	Days After Application	Milligrams Oil per Gram of Twig		
				Range	Average	Per Cent Decrease
1-A	Dormant oil, 205 vis. (with lime sulfur)	10.8	1	1.3–2.5	1.9	—
1-B	Dormant oil, 205 vis. (with lime sulfur)	10.8	1	2.1–3.0	2.5	—
2	Dormant oil, 205 vis. (with lime sulfur)	9.2	1	2.4–3.2	2.8	—
			7	1.3–2.7	2.1	25
3	Dormant oil, 205 vis. (with dinitrocresol)	7.9	1	1.7–1.9	1.8	—
4	Dormant oil, 205 vis.	8.0	1	1.2–1.9	1.6	—
			14	1.0–1.4	1.2	25
5	Dormant oil, 405 vis.	7.3	1	1.9–2.6	2.2	—
			15	1.4–2.1	1.8	18
6	Polybutene	8.4	1	1.3–1.6	1.4	—
			15	1.2–1.8	1.4	0

[a] Applied March 22 except plot 2 on March 31.

The following spray chemicals were used: Dormant spray oil (unsulfonated residue 75%, viscosity 205 seconds Saybolt at 100° F.), experimental dormant oil (unsulfonated residue 77%, viscosity 405 seconds Saybolt at 100° F.), polybutene Indopol H-100 (viscosity 44,000 seconds Saybolt at 100° F.), lime sulfur concentrate, 50% dinitrocresol wettable powder, Soya flour emulsifier, non-ionic polyglycol surfactant (Dynawet), and non-ionic alkyl aryl polyether alcohol surfactant (Triton X-114).

The dormant application was made on March 22 when the bud scales had barely begun to separate; the delayed dormant application was made 9 days later when the leaf tips were showing. When the dormant spraying was done, adult psylla were numerous and an occasional egg could be found. At the time of the delayed dormant application, there were more than 15 eggs per fruit spur on the non-sprayed check plot.

The standard (205 vis.) dormant oil was emulsified with Soya flour at 1 pound per acre to provide a quick-breaking emulsion. The experimental oil (405 vis.) was emulsified with Dynawet at 1.2 pints per acre. The polybutene was emulsified with Triton X-114 at the rate of 1.2 pints per acre. Since the amount of spray liquid that was applied per acre was insufficient to produce any drip, presumably the "tightness" of the oil emulsions had no influence on oil deposition.

The procedure in sampling the plots for oil deposit analysis was to sample at random, from the 5- to 7-foot level, 25 twigs per tree. The second series of determinations required 50 twigs per tree because of lower oil deposits. Five trees were sampled per plot at each sampling date. The twigs were cut to a standard length of 10 cm., were weighed, and the oil deposits were then related to the weight of the twigs as milligrams per gram.

The control obtained by the various spray mixtures was estimated by counting the numbers of psyllid eggs and nymphs on 4 fruit spurs from each of 5 trees per plot at approximately weekly intervals until the leaves had developed. Thereafter 20 leaves were sampled from each of 5 trees per plot. The samples were taken to the laboratory and the number of eggs or nymphs counted with the aid of stereomicroscopes. When the psyllids became numerous enough to necessitate summer spray treatment, the experiment was discontinued. An approximation of the numbers of adults was obtained by two observers, each walking around half of a tree, who recorded the number of psyllids they could see in 2 minutes. The observers examined five trees in the center of each plot.

EXPERIMENTAL RESULTS.—*Oil Deposits.*—The intended amount of oil per acre was 7.2 gallons, but this amount was exceeded in every plot and in the oil-lime sulfur plots, widely exceeded. However, the oil deposit determinations show the quantities of oil that actually were present on the twigs. Table 1 gives the initial oil deposits and the percentage loss at the sampling intervals. The data suggests that the 205 vis. oil deposit declined more rapidly than the 405 vis. oil. The remarkable persistence of polybutene, obvious enough from the appearance of the sprayed trees, is evident in that there was no loss of deposit after 15 days' exposure.

Although plots 1A and 1B were sprayed from the same tank, there is a considerable difference in initial deposit. Since plot 1B was sprayed after plot 1A, this may be an indication of the instability of the quick-breaking oil emulsion. There was no indication of phytotoxicity from any of the spray mixtures.

Numbers of Pear Psyllids and Their Eggs.—The numbers of psyllid eggs on the twigs was determined for a 5-week period after spraying (table 2). Oil and dinitrocresol gave excellent control, and but few eggs were found after treatment. The addition of lime sulfur to dormant oil resulted in an increase of psyllid eggs as compared with the oil alone. Treatment at the delayed dormant stage allowed an initially heavy deposition of eggs as compared with dormant spraying but a reduced deposition 3 weeks to a month later. Evidently the standard 205 vis. dormant oil was less effective in preventing egg deposition

Table 2.—Abundance of pear psylla eggs following applications of dormant and delayed dormant sprays. Summerland, B. C. 1960.

Materials	Dosage per Acre	Average No. of Eggs per Fruit Spur				
		March 29	April 6	April 13	April 20	April 27
205 vis. dormant oil + lime sulfur[a]	10.8 gal. + 21.6 gal.	1.3	4.6	6.6	11.0	23.6
205 vis. dormant oil + lime sulfur[a]	9.2 gal. + 20.4 gal.	—	16.6	25.6	9.0	5.3
205 vis. dormant oil + dinitrocresol 50%	7.9 gal. + 4 lb.	2.4	2.4	0.4	0.5	0.3
205 vis. dormant oil	8.0 gal.	4.0	2.9	5.1	11.6	9.9
405 vis. dormant oil	7.3 gal.	2.8	2.9	1.7	4.8	4.9
Polybutene H-100	8.4 gal.	2.1	2.3	6.9	16.4	16.5
Untreated	—	15.7	25.0	37.9	64.7	73.5

[a] Applied at delayed dormant stage, March 31. All other treatments applied at dormant stage, March 22.

than the more viscous experimental oil. The polybutene at first appeared to be as effective as the experimental oil but within 3 weeks began to fail. The polybutene plot adjoined the check plot which was heavily infested, and this may explain the increase in egg deposition.

Table 3 shows the numbers of overwintered psyllids that were present after the dormant spray applications. Taking into consideration that the oil deposit on the oil-lime sulfur plot was approximately the same as on the plot sprayed with oil alone, there is an indication that the presence of lime sulfur did not make the spray deposit more repellent, or more toxic, to the pear psylla. On the other hand, the smaller number of adult psyllids in the oil-dinitrocresol plot is noteworthy because the oil deposit in that plot was not particularly high.

Table 3.—Numbers of overwintered adult pear psyllids after application of dormant and delayed dormant sprays. Summerland, B. C. 1960.

Materials	Dosage per Acre	Total Adults per 5 Trees	
		March 30[a]	April 7[b]
205 vis. dormant oil + lime sulfur	10.8 gal. + 21.6 gal.	216	92
205 vis. dormant oil + lime sulfur[c]	9.2 gal. + 20.4 gal.	—	39
205 vis. dormant oil + dinitrocresol 50%	7.9 gal. + 4 lb.	42	5
205 vis. oil	8.0 gal.	183	61
405 vis. oil	7.3 gal.	91	43
Polybutene H-100	8.4 gal.	72	33
Untreated	—	183	109

[a] Eight days after treatment.
[b] Fifteen days after treatment.
[c] Applied at delayed dormant stage, March 31. All other treatments applied at dormant stage, March 22.

Incidentally, the dormant oil-lime sulfur mixture, by spotting the wings of the psyllids, served to label them and gave some evidence concerning the longevity of the insects after they were sprayed and of their flight habits. The ratio of spray-spotted psyllids to nonspotted ones was as great 2 weeks after treatment as a week after treatment. This suggests, as Ross (1932) has stated, that 205 vis. oil is not effective in killing the overwintered insects. Some spray-spotted psyllids were seen in trees that were 17 rows, i.e., 340 feet, removed from the plots in which the insects had been sprayed with the oil-lime sulfur mixture. Data given in table 4 show that on June 6 it became necessary to apply a summer spray to the dormant and delayed dormant oil-lime sulfur, polybutene, and check plots. On May 30, 69 days after the dormant sprays had been applied, the infestation in those treated plots became as heavy as in the untreated check plot.

On the other hand, it was possible to allow the infestation to develop until July 27 in the plots that had been treated with 205 vis. oil alone, 405 vis. oil, and oil-dinitrocresol. Presumably, because of the dispersal of the psyllids, the infestation in the oil-dinitrocresol plot, which had been exceptionally low early in the season, became as heavy by mid-June as in the neighboring plots that had been treated with dormant oil alone. This also appeared to occur in the plot sprayed with 405 vis. oil. The nymphal counts were continued through July, and the plots showed a steady increase until after July 5, when the numbers of psyllid nymphs sharply dropped. Two factors seemed to be responsible for the reduction. One was the predatory action of *Anthocoris melanocerus* Reuter, which in British Columbia appears to be an effective agent of biological control (Madsen 1961). The other was a period of extremely hot weather in which many drops of honeydew became solidified and nymphal mortality was high. This phenomenon has been reported in British Columbia by Marshall (1959). Since it was no longer feasible to attribute the population fluctuations to the effect of the spray treatments, the plots were discontinued.

There seem to be three reasons for the unusual effectiveness of the oil-dinitrocresol mixture. The data in table 3 show it to be toxic to the adult pear psylla, and it is in all likelihood toxic to the eggs. In addition, the presence of dormant oil in the mixture has a deterrent effect upon oviposition. There is another consideration: the pear psylla has shown exceptional capacity to develop resistance to insecticides; but as far as can be learned, no resistant strains of insects have been selected with respect to either petroleum oils or to dinitrocresol.

The oil-dinitrocresol mixture may, however, have an unwelcome characteristic the full significance of which cannot yet be assessed. Early in the season, *Anthocoris melanocerus* was observed less frequently in the plots sprayed with oil-dinitrocresol than any of the others. Observations in July, however, did not show any difference in anthocorid numbers in the plots treated with the 205 vis. oil, 405 vis. oil, or the oil-dinitrocresol.

From the data obtained thus far, it may prove possible to control the pear psylla in British Columbia with a

Table 4.—Numbers of pear psylla nymphs on fruit spurs and leaves following dormant and delayed dormant treatments. Summerland, B. C. 1960.

Materials	Dosage per Acre	Nymphs per 20 Fruit Spurs				Nymphs per 100 Leaves					
		April 13	April 20	April 27	May 6	May 20	May 30	June 6	June 20	July 5	July 26
205 vis. dormant oil + lime sulfur	10.8 gals. 21.6 gals.	10	3	47	115	526	637	—[a]	—	—	—
205 vis. dormant oil + lime sulfur[b]	9.2 gals. 20.4 gals.	11	34	103	99	143	277	—[a]	—	—	—
205 vis. dormant oil + dinitrocresol 50%	7.9 gals. 4 lbs.	1	0	6	6	21	5	24	233	508	84
205 vis. dormant oil	8.0 gals.	11	2	30	80	147	166	61	201	306	80
405 vis. dormant oil	7.3 gals.	6	2	15	79	62	95	31	180	237	86
Polybutene H-100	8.4 gals.	2	3	19	77	115	159	—[a]	—	—	—
Untreated	—	51	98	224	625	925	466	—[a]	—	—	—

[a] Resprayed prior to this sampling date.
[b] Applied at delayed dormant stage, March 31. All other treatments applied at dormant stage, March 22.

single spray treatment early in the spring. The success of such a program will depend upon the ability of the pear psylla to reinfest treated orchards and upon the effect exerted by natural enemies and climatic factors during the summer period.

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Experiments with Insecticides for Control of the Green Peach Aphid on Potatoes[1]

RALPH SCHOPP, B. J. LANDIS and G. T. HAGEL,[2] *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

After the green peach aphid (*Myzus persicae* (Sulzer)) became tolerant to parathion, field-plot experiments were begun in 1954 to try to find a more effective insecticide. Both multiple and single applications were tested. Endrin and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) gave better control than parathion, but Thiodan was the most effective.

The problem of controlling the green peach aphid (*Myzus persicae* (Sulzer)) on potatoes in the Pacific Northwest, where it is an important vector of the virus causing leaf-roll disease, appeared near solution when parathion became available in 1950. However, beginning in mid-season in 1952 previously effective dosages of parathion and other organophosphorus insecticides gave unsatisfactory control.

METHODS AND MATERIALS.—From 1954 to 1959 two types of experiments were used in testing the effectiveness of insecticides applied with power equipment in the Yakima Valley of Washington. The first consisted of multiple applications designed for maintaining a low aphid infestation during the production of the crop, and the second of single applications to evaluate a large number of new insecticides. The multiple-application experiments were on plots 8 to 16 rows wide and 100 to 600 feet long and single-application experiments on plots four rows wide and 100 to 200 feet long. Dusts and sprays were

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applied with tractor-mounted row-crop dusters and sprayers that covered swaths four rows wide. Treatments were replicated four to eight times in randomized blocks. Their effectiveness was determined by counting the aphids on 25 to 50 compound leaves from each plot picked at random from the lower half of the plants. Leaf samples were collected at intervals, usually 3, 7, and 14 days after treatment. There were no untreated check plots, but parathion or mixtures of parathion with DDT and sulfur were used for comparison.

CHEMICAL NAMES OF THE PROPRIETARY MATERIALS TESTED

Am. Cyanamid 18706—*O,O*-dimethyl *S*-(ethyl carbamoylmethyl) phosphorodithioate
Bulan—1,1-bis(*p*-chlorophenyl)-2-nitrobutane
Delnav®—2,3,*p*-dioxanedithiol *S,S*-bis (*O,O*-dimethyl phosphorodithioate)
Diazinon®—*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dibrom®—1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Di-Syston®—*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate
Guthion®—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate
Monsanto CP-10502—1-(dimethoxyphosphinyl)vinyl dimethyl phosphate
Phosdrin®—1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate
Rohm & Haas C-140—*O*-(dimethyl aminomethyl) phenyl ester hydrochloride of dimethyl carbamic acid
Sevin®—1-naphthyl *N*-methylcarbamate
Thiodan®—6,7,8,9,10,10,-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Trithion®—*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate

MULTIPLE APPLICATIONS.—In these experiments various new insecticides as well as increased dosages of parathion were tested. Because of the large numbers of aphids in continuous flight to potato fields and their rapid reproduction on the crop, several applications of most insecticides were required each season. However, definite schedules were difficult to maintain because of conflicts with growers' irrigation practices. Sprays were applied at 12 gallons and dusts at 25 pounds per acre.

The periods when the development of aphid populations was sufficient to show measurable differences in control with insecticides varied from year to year. For this reason, it was necessary some years to consider only the samplings from the multiple application experiments that were taken during the period of appreciable aphid productivity.

In 1954 eight dusts were applied five times—May 13 and 24, June 4 and 18, and July 6—on an early-crop field of potatoes near Toppenish. The field became infested with aphids very slowly in May and June, apparently because the peach orchards, the source of spring migrations, were approximately 10 miles away. In the latter part of June there was a heavy migration from weeds, and thereafter the population increased rapidly on the potatoes. Counts were made from May 17 to July 27, but only those made 3, 7, and 14 days after the last application are included in the averages given in table 1. The early counts showed few aphids present, and by July 27 the differences between the treatments had become obscured by reinfestation. The standard parathion-DDT-sulfur dust had been widely used for 2 years by potato growers for the control of aphids and the tuber flea beetle (*Epitrix tuberis* Gentner).

Fairly effective control of the aphids was obtained with all dusts except Dilan (2-nitro 1,1-bis(*p*-chlorophenyl) propane and butane mixture (1:2 mixture), not included in table, until the last week of July, when the crop was harvested. The Diazinon-sulfur dust gave the most effective control for the first week, but thereafter was less effective than the parathion-DDT-sulfur or parathion-dieldrin-sulfur. Parathion-cryolite was not significantly more effective than parathion-DDT. When only ¼ pound of parathion was applied, mixtures with endrin or dieldrin

Table 1.—Control of the green peach aphid on potatoes with multiple applications of insecticides.

INSECTICIDE	ACTIVE INGREDIENTS PER ACRE (LB.)	NO. OF APHIDS PER 1,000 LEAVES
1954 (5 applications, 5 replicates)		
Dusts		
Parathion+DDT+sulfur[a]	0.25+1.25+6.25	135
Parathion+cryolite+sulfur	.25+8.25+6.25	83
Parathion+dieldrin+sulfur	.25+0.25+6.25	35
Parathion+endrin+sulfur	.25+ .25+6.25	23
Parathion+DDT+sulfur	.5 +1.25+6.25	14
Diazinon+sulfur	1.0 +6.25	20
Endrin	0.25	32
L.S.D. @ 5% level	—	55
1955 (2 applications, 5 replicates)		
Dusts		
Endrin	0.25	178
	.5	134
Parathion+sulfur[a]	.25+6.25	84
Diazinon+sulfur	1.0 +6.25	54
Sprays		
Diazinon	1.0	102
Parathion	0.5	84
Endrin	.5	22
L.S.D. @ 5% level	—	92
1956 (3 applications, 4 replicates)		
Dusts		
Parathion+sulfur[a]	0.25+6.25	142
	.5 +6.25	32
Diazinon+sulfur	.5 +6.25	131
	1.0 +6.25	0
Endrin	0.25	38
	.5	8
Sprays		
Parathion	0.5	86
Diazinon	1.0	23
Endrin	0.5	7
L.S.D. @ 5% level	—	78
1959 (3 applications, 8 replicates)		
Sprays		
Parathion[a]	1.0	613
Phorate	0.5	563
Endrin	1.0	234
Thiodan	1.0	72
L.S.D. @ 5% level	—	366

[a] Standard treatment.

Table 2.—Control of the green peach aphid on potatoes with single applications of insecticides.

Date of Application	Insecticide	Active Ingredient per Acre (lb.)	No. of Aphids per 100 Leaves			
			Before Treatment	Days After Treatment		
				3	7	14
1957						
May 31	Phosdrin	2.0	350	4	21	71
	Demeton	0.5	323	49	65	96
	Di-Syston	.5	321	25	47	147
	Thiodan	.5	363	21	60	155
	Parathion[a]	.5	371	10	72	171
	Phorate	.5	273	63	99	236
	Trithion	.5	382	49	87	291
	Guthion	.5	359	89	297	368
	Dicapthon	.5	273	51	183	613
	Malathion	1.0	276	211	416	691
	L.S.D. @ 5% level	—	—	49	83	293
June 20	Demeton	0.5	19	3	4	67
	Thiodan	.5	37	3	25	127
	Parathion[a]	.5	29	2	24	183
	Phosdrin	.5	20	1	19	214
	Malathion	1.0	13	4	19	239
	Phorate	0.5	15	7	17	241
	Trithion	.5	20	9	16	274
	Guthion	.5	16	40	59	404
	L.S.D. @ 5% level	—	—	21	31	217
July 2	Demeton	0.5	228	51	243	—
	Thiodan	.5	296	16	347	—
	Trithion	.5	230	98	386	—
	Phosdrin	.5	184	23	408	—
	Phorate	.5	152	62	477	—
	Parathion[a]	.5	189	63	569	—
	Malathion	1.0	173	165	664	—
	Dicapthon	0.5	198	277	793	—
	Guthion	.5	129	261	838	—
	DDT	.5	171	327	1,083	—
	L.S.D. @ 5% level	—	—	96	259	—
1958						
June 10	Thiodan dusts[b]					
	Alone	0.8	1,889	217	271	23
	With sulfur	.8	1,576	78	233	33
	Thiodan					
	Miscible	.3	1,713	281	337	24
	Oil Emulsion	.3	1,655	623	732	35
	Endrin	.3	1,720	505	366	43
	Parathion[a]	.3	1,620	273	1,360	680
	L.S.D. @ 5% level	—	—	293	225	226
June 23	Di-Syston	0.4	1,013	56	107	27
	Rohm & Haas C-140	.4	896	62	40	45
	Phosphamidon	.4	1,237	245	326	89
	Parathion[a]	.4	981	141	276	107
	Dibrom	.4	818	362	457	107
	Ronnel	.4	1,134	480	384	218
	L.S.D. @ 5% level	—	—	242	209	—
July 21	Di-Syston	0.4	24	5	1	4
Experiment 1	Monsanto CP-10502	.4	36	5	36	7
	Rohm & Haas C-140	.4	39	4	5	9
	Sevin	.4	43	5	37	15
	Endrin	.4	33	0	49	16
	Endrin+Sticker	.4	50	2	9	24
	Am. Cyanamid 18706	.4	36	13	8	26
	Ronnel	.4	21	9	32	27
	Delnav	.4	52	13	33	27
	Phosdrin	.4	28	8	13	32
	Parathion[a]	.4	32	7	7	39
	Dimethoate	.4	19	4	21	44

(*Continued on next page*)

[a] Standard treatment.
[b] All sprays except for two Thiodan dusts applied on June 10, 1958.

Table 2.—(*Continued*)

Date of Application	Insecticide	Active Ingredient per Acre (lb.)	No. of Aphids per 100 Leaves			
			Before Treatment	Days After Treatment		
				3	7	14
July 21	Demeton	0.4	24	1	2	4
Experiment 2	Dibrom	.8	23	5	8	6
	Phosphamidon	.8	22	7	4	8
	Diazinon	.4	13	2	10	11
	Bulan	.8	31	10	24	15
	Parathion[a]	.4	25	5	5	30
	Thiodan+dipropylene glycol	0.4	17	2	8	2
	Thiodan+Sticker	.4	13	1	7	4
	Thiodan (1957 Batch)	.4	25	1	4	5
	Thiodan (1958 Batch)	.4	32	1	4	5
	Thiodan+Sesamex	.4	25	0	1	9
	Thiodan (Oil emulsion)	.4	27	4	3	11
1958				*5 days*	*8 days*	*20 days*
July 9	Rohm & Haas C-140	0.4	—	131	70	14
	Ronnel	.4	—	517	696	23
	Dibrom	.4	—	347	408	26
	Di-Syston	.4	—	55	81	31
	Monsanto CP-10502	.4	—	287	455	35
	Am. Cyanamid 18706	.4	—	181	217	75
	Parathion[a]	.4	—	165	422	101
	Dimethoate	.4	—	144	125	211
	Bulan	.4	—	522	648	333
	Phosphamidon	.4	—	349	372	503
	L.S.D. @ 5% level	—	—	193	171	193

were most effective, but they were less effective than the mixture with DDT when ½ pound of parathion was used.

In 1955 four dusts and three sprays were tested on a field near Harrah, Wash. This field was much closer to the orchard district than the one used in 1954, but again few aphids were found on the plants in June and July. The plots were treated on June 11 and July 19, and seven counts were made between June 9 and July 27. Only counts made 3 and 7 days after the last application are included in table 1, because practically no aphids were found in the early counts. A large part of the field outside the experimental tract was not treated and had few aphids at any time.

Analysis of the data indicated one-fourth pound of endrin might be inferior to the standard, but the difference required for significance was large because of the erratic infestation within the field and some other treatments with fewer aphids than the standard were not significantly different for this reason.

In 1956 six dusts and three sprays were applied three times—on May 21 and June 6 and 28—on an early-crop field of potatoes near Toppenish, Wash. Nine counts were made from May 21 to July 3, but only those made 3 and 7 days after each application are included in table 1. After the seventh day the differences between treatments were becoming obscured by immigrating aphids.

Diazinon was very effective for a week and was better as a dust than as a spray. Of particular concern, however, was the slow rate at which lady beetles re-entered the Diazinon-treated plots to feed on newly established aphid colonies. All the treatments were significantly better than the standard except the Diazinon dust which was about equal to the standard. Endrin was effective over a longer period than either parathion or Diazinon.

These three experiments showed that, although the one-fourth pound of parathion could be made more effective by adding an equal amount of either dieldrin or endrin, the most effective results were obtained by increasing the amount of parathion to one-half pound per acre. Except in 1955, when the results were inconclusive, endrin was more effective than parathion and also over a longer period. Diazinon at 1 pound per acre was very effective for a week, after which most of its effects disappeared. This was apparently due to its short residual effect and the destruction of natural enemies of the aphids, such as lady beetles.

In 1959 sprays containing Thiodan, endrin, and phorate were compared with the parathion standard in a field near Brownstown. The plots were sprayed three times—June 26 and July 8 and 20. Eight counts were made, but only those made 3, 7, 14, and 21 days after the last application are included in the averages. The plots were 16 rows wide to take advantage of the fumigation action these sprays were thought to have. Thiodan and endrin were significantly better than parathion, but the margin with Thiodan was much larger than with endrin. Phorate at one-half pound per acre was about equal to the parathion standard.

SINGLE APPLICATIONS.—In these experiments the insecticides were applied as sprays, except where dusts are indicated. Most of the materials were tested at one rate (table 2), but some were tested at three (table 3).

The data for 1957 (table 2) indicate that demeton, Phosdrin, Di-Syston, and Thiodan were more effective

than parathion, but were not shown to be significantly so. Trithion and phorate appeared inferior to parathion, but more effective than malathion, dicapthon, Guthion, and DDT.

In the 1958 experiments Di-Syston, demeton, Thiodan, and Rohm & Haas C-140 appeared superior to parathion. C-140 was not available in 1957, but the others were among the most effective four tested in that year also. Dimethoate gave good control 3 days after application, but was very ineffective after 2 weeks. Am. Cyanamid 18706 did not give such good immediate control but had some residual effect at 14 days. Large numbers of dead lady beetles were noted in the plots sprayed with dimethoate, and elimination of these predators may have allowed more rapid reinfestation. The systemic insecticides demeton and Di-Syston were effective for about 2 weeks and were among the most satisfactory in these tests. Rohm & Haas C-140 and Thiodan also had long residual effects, although they are apparently not systemic. Monsanto CP 10502, Dibrom, and phosphamidon were not very effective, and results with Bulan, ronnel, Sevin, and Delnav were very unsatisfactory.

Thiodan was tested as dust alone and with sulfur on June 10. In one treatment in which $6\frac{1}{2}$ pounds per acre of sulfur was included fewer aphids were present after 3 days than when the dust contained no sulfur. However, on the seventh day there was little difference between them.

Six Thiodan sprays tested on July 21 gave about the same results. Of two emulsive materials tested an almost black German product obtained in 1957 was as effective as an amber-colored American product obtained in 1958. Thiodan was not improved by incorporating it in an emulsifiable oil, or by adding a dipropylene glycol stabilizer, the synergist sesamex, or a wax dispersion sticker, but all these preparations resulted in fewer aphids than the parathion standard.

Thiodan, Phosdrin, Trithion, and phorate were also compared with parathion at three dosages (table 3). The insecticides were applied when enormous numbers of aphids were migrating into the field. Thiodan was the most effective. Phosdrin and Dibrom reduced the aphid populations greatly by the third day, but by the seventh day much of their toxicity had been lost. Trithion and phorate were not effective at the dosages used.

DISCUSSION.—Demeton, Di-Syston, Thiodan, and Rohm & Haas C-140 gave good control of the green peach aphids on potatoes in foliage sprays applied with ground equipment. Demeton and Di-Syston are systemic insecticides and residues could be troublesome when used at rates adequate for aphid control. Thiodan and Rohm & Haas C-140 in sprays were very effective and have long residual effects, although they are not systemics. The manufacturer is no longer making C-140, and this insecticide will probably not be available commercially.

Thiodan emulsive spray appears to be the most practical insecticide for control of the green peach aphids in the Pacific Northwest. An oil emulsion was less effective than the emulsive concentrate spray; a dust required considerably more actual Thiodan for comparable results. The effectiveness of sprays was not improved perceptibly by the addition of a dipropylene glycol stabilizer, sesamex, or a wax dispersion sticker.

Endrin was generally inferior to Thiodan but gave fairly good control. It has a long residual effect and is readily available. Diazinon and Phosdrin gave good immediate kill of the aphids, but posttreatment population trends indicated that frequent applications were required.

Table 3.—Control of the green peach aphid on potatoes with single applications of different dosages of insecticides in sprays.

Date of Application	Insecticide	Active Ingredient per Acre (lb.)	No. of Aphids per 100 Leaves		
			Before Treatment	Days After Treatment	
				3	7
1957					
July 15[a]	Thiodan	2.0	4,395	19	279
		1.0	4,452	171	1,905
		0.5	3,755	247	1,749
	Parathion	2.0	4,589	88	733
		1.0	4,708	840	1,888
		0.5	4,314	453	2,809
	Trithion	4.0	3,891	455	924
		2.0	5,351	1,026	2,715
		1.0	5,276	1,179	3,266
	Phosdrin	2.0	4,756	52	1,215
		1.0	4,115	121	1,744
		0.5	3,666	548	3,514
	L.S.D. @ 5% level		—	746	1,767
July 16[a]	Parathion	2.0	5,682	107	918
		1.0	5,077	552	3,141
		0.5	5,243	566	4,416
	Dibrom	2.0	5,966	51	1,293
		1.0	5,288	299	2,491
		0.5	5,307	1,429	7,556
	Phorate	4.0	5,674	1,277	9,908
		2.0	7,564	1,714	8,778
		1.0	5,241	2,522	16,114
	L.S.D. @ 5% level		—	795	3,155

[a] Six replicates.

Laboratory Toxicity of Several Insecticides to the Honey Bee[1]

C. A. JOHANSEN,[2] *Washington State University, Pullman*

ABSTRACT

LD_{95}'s for honey bees (*Apis mellifera* L.) held 72 hours after mist chamber treatments were as follows: parathion<Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate)<dieldrin<Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate)<phosphamidon<Sevin® (1-naphthyl *N*-methylcarbamate)<phorate<Trithion® (S-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)<DDT<ethion<Phostex® (bis(dialkoxyphosphinothioyl) disulfides (alkyl ratio 25% isopropyl, 75% ethyl). The first five materials had a mean LD_{95} of 0.0046%; the next four materials, 0.039%; and the last two materials, 0.57% concentration. These apparent levels of toxicity determined in the laboratory are not necessarily indicative of bee poisoning hazard in the field where residual action is an important factor. Bioassay of laboratory-killed honey bees showed 1.0 microgram of dieldrin per bee; while field-killed bee samples contained 0.5 microgram of dieldrin per bee.

A number of insecticidal compounds were tested for toxicity to honey bees (*Apis mellifera* L.) in the laboratory during 1957–59. Since Anderson and his associates had already started a laboratory testing program using dust formulations of pesticides, it was decided to base these investigations of honey bee toxicity upon sprays (see Anderson & Tuft 1952, Anderson & Atkins 1954).

MATERIALS AND METHODS.—Insecticides were tested in a small mist chamber consisting of a glass cylinder ($11\frac{1}{4}$ inches inside diameter and $14\frac{3}{4}$ inches high) with a hardboard cover. A brass atomizer was attached to the hardboard so that the nozzle orifice was $12\frac{1}{8}$ inches above the bottom of the chamber.

Honey bees were placed in disposable cone-shaped cages constructed from 18-mesh wire screen. The diameter of the base of the cone was $6\frac{3}{4}$ inches and the height to a $\frac{3}{4}$-inch-diameter opening was $4\frac{3}{4}$ inches. Approximately 25 honey bees were loaded into each cage for the tests and the opening closed with a cork.

All insecticides were diluted with analytical grade acetone; the controls were sprayed with acetone only. Acetone had no adverse effect upon check bees so long as it was analytical grade. Two ml. of spray were drawn from a test tube in each application. Ten pounds air pressure was maintained in the atomizer system throughout the application. Each cage was removed from the mist chamber 1 minute after all of the liquid had been drawn from the test tube. Spraying was conducted inside a fume-hood. Parathion standards were run periodically to check the reproducibility obtained with the methods employed.

After removal from the mist chamber, the cork was removed from each cone cage and replaced with a wad of cotton soaked in 1:1 sugar-water syrup. Depressions were formed in the tops of the cotton feeders, and these were moistened once per day. All test cages of bees were held in a controlled temperature cabinet at 72° F. and mortality counts were taken at 72 hours after application. The criterion of death was lack of any movement, even after stimulation. Seventy-two hours was chosen as the test duration because residual action and slow toxic action are important factors in the bee poisoning hazard of pesticides.

Each test series was corrected for the check by means of Abbott's formula. Mortality for the controls usually varied from 0% to 5%; tests with controls of greater than 5% mortality were discarded. The average mortality data for four replications of each dilution of an insecticide were plotted on log-probit paper.

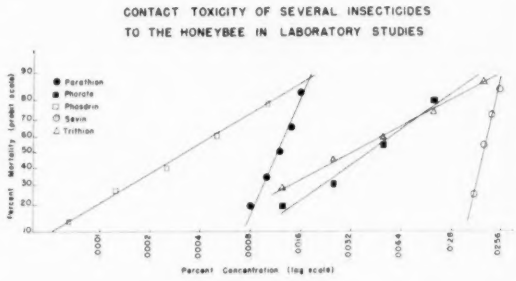

FIG. 1.—Dosage-mortality curves for five insecticides to the honey bee, Pullman, Washington, 1957–58.

RESULTS.—Mortality curves obtained for a number of insecticides are presented in figures 1 and 2. LD_{50}'s and LD_{95}'s are given in table 1.

Note that three levels of inherent contact toxicity are distinguishable as represented by the LD_{95}'s (better criteria of actual field hazard than LD_{50}'s). These levels are separated by approximately 10-fold differences. Parathion, Phosdrin®, dieldrin, Guthion®, and phosphamidon fall in the high toxicity group with a mean LD_{95} of 0.0046% concentration; Sevin®, phorate, Trithion®, and DDT fall in the moderate toxicity group with a mean LD_{95} of 0.039% concentration; and, ethion and Phostex® fall in the low toxicity group with a mean LD_{95} of 0.57% concentration.

These inherent contact toxicity ratings in the laboratory do not necessarily indicate the bee poisoning hazard in field use. For example, experience shows that Sevin is much more hazardous to honey bees than Phosdrin because the former possesses long residual action, while the latter has very short residual action (Johansen 1960).

[1] Scientific Paper 2102. Washington Agricultural Experiment Stations. Work conducted under Project No. 1419. Presented at the meeting of the Pacific Branch of the Entomological Society of America held at Spokane, Washington, June 27–30, 1960. Accepted for publication April 17, 1961.

[2] The author gratefully acknowledges the assistance of J. S. Waterhouse, present address Dept. Science & Mathematics, State University Teachers College, Plattsburg, N. Y., and Richard Welton, currently a Graduate Assistant at Oregon State University, Corvallis, during portions of the investigations. R. F. Harwood and R. L. Payne, Associate Entomologist and Scientific Aide, respectively, Washington State University, conducted the bioassays of honey bee samples reported in this paper.

Table 1.—Lethality of insecticides applied as sprays to honey bees in laboratory tests, Pullman, Washington, 1957–59.

MATERIAL	LD_{50} (% CONC.)	MATERIAL	LD_{95} (% CONC.)
Phosdrin	0.0003	Parathion	0.002
Parathion	0.002	Phosdrin	0.004
Dieldrin	0.003	Dieldrin	0.005
Trithion	0.003	Guthion	0.006
Phorate	0.004	Phosphamidon	0.007
Phosphamidon	0.005	Sevin	0.028
Guthion	0.005	Phorate	0.031
Sevin	0.020	Trithion	0.047
DDT	0.029	DDT	0.048
Ethion	0.078	Ethion	0.319
Phostex	0.151	Phostex	0.823

ANALYSES OF LABORATORY- AND FIELD-TREATED HONEY BEES.—Approximately 1,000 honey bees were treated in groups of 50, following the standardized laboratory procedure presented above. Each caged group was sprayed with 2 ml. 0.005% dieldrin, the previously determined LD_{95} at 72 hours shown in table 1. Approximately a 95% mortality did result and the dead bees were placed in frozen storage 72 hours after treatment.

Honey bees were also collected in dead-bee pans with double-screen tops and louvered false-bottom drawers (type developed by F. E. Todd, U. S. Bee Culture Laboratory, Tucson, Arizona), and traps (type figured in Johansen, 1960a) attached to hives placed in an alfalfa field treated with 0.5 lb. dieldrin per acre. The hive landing boards were protected from spray drift with polyethylene tarpaulin during treatment and the dead-bee pans were not attached to the hives until after the application was made. Field samples of dead bees were collected from the traps 24 hours after the application and held in frozen storage.

Drosophila bioassays showed that the laboratory-treated samples contained 1.0 microgram of dieldrin per bee; while the field-treated bees were calculated to contain 0.5 microgram of dieldrin per bee.

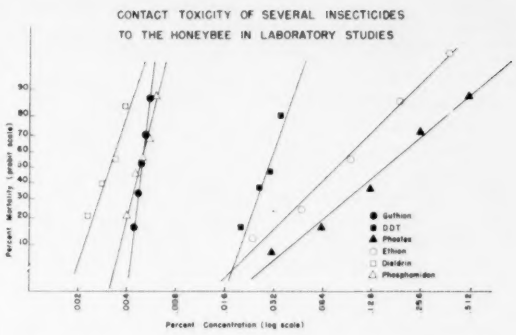

FIG. 2.—Dosage-mortality curves for six insecticides to the honey bee, Pullman, Washington, 1959.

CHEMICAL DEFINITIONS.—The chemical definitions of the insecticides mentioned in this paper that do not have adopted common names are:

Guthion®, *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate.

Phosdrin®, 1-methoxycarbonyl-1-propene-2-yl dimethyl phosphate.

Phostex®, bis(dialkoxyphosphinothioyl) disulfides (alkyl ratio 25% isopropyl, 75% ethyl).

Sevin®, 1-naphthyl *N*-methylcarbamate.

Trithion®, S-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.

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Evaluation of Adhesives for Sticky Board Traps[1]

GEORGE H. KALOOSTIAN, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Yellow, 5- ×10-inch sticky-board traps were exposed in a peach orchard from August 29 to September 28, 1960 to compare Deadline®, Tanglefoot®, and Stickem® as coating materials. Boards coated with Deadline and Stickem captured 10 times as many Homoptera (Cicadellidae) and Diptera as those coated with Tanglefoot. Stickem is suggested as a substitute for Deadline, which is no longer available commercially.

Because of their simplicity, sticky traps have been eminently effective in insect survey activities and as a research tool for collecting biological data about certain insects. More than 200 references exist in the literature on the use of adhesives for catching insects. Until about 25 years ago the material most universally used was Tanglefoot®[2]. Adhesive-coated paper, muslin, strings, wire screens, pegs, and boards in different sizes, shapes, forms, and colors were devised mainly to control insects. Then the push-button age provided help with the announcement of an aerosol dispenser for the application of a sticky compound for the control of crawling insects (Anon. 1960).

Forbush & Fernald (1896) described the "Shaw moth-

[1] Accepted for publication April 25, 1961.

[2] Deadline®, Tanglefoot®, and Stickem® were purchased from the following firms, respectively: California Spray Chemical Corp., Richmond, Calif.; The Tanglefoot Co., Grand Rapids, Mich.; and Michel & Pelton, Oakland, Calif. The products and manufacturers are not presented as a complete list and their mention herein does not imply any guarantee or endorsement by the U. S. Department of Agriculture.

Table 1.—Comparison of the number of Cicadellidae and Diptera collected by traps coated with Deadline, Tanglefoot, or Stickem. August-September 1960.

Adhesive	Trap No.	Number of Days Traps Exposed								
		10	20	30	10	20	30	10	20	30
		Cicadellidae			*Diptera*			*Total Insects*		
Deadline	1	23	12	35	105	271	376	128	283	411
	2	30	25	55	137	174	311	167	199	366
	3	64	49	113	117	220	337	181	269	450
	4	48	21	69	109	232	341	157	253	410
	5	33	41	74	121	173	294	154	214	368
Totals		198	148	346	589	1,070	1,659	787	1,218	2,005
Tanglefoot	1	11	1	12	12	11	24	24	12	36
	2	11	6	17	6	8	14	17	14	31
	3	12	5	17	4	15	19	16	20	36
	4	10	7	17	3	7	10	23	14	27
	5	12	13	25	3	16	19	15	29	44
Totals		56	32	88	29	57	57	85	89	174
Stickem	1	51	53	104	102	264	366	153	317	470
	2	35	29	64	100	197	297	135	226	361
	3	25	17	42	62	137	199	87	154	241
	4	33	27	60	114	135	249	147	162	309
	5	56	50	106	136	171	307	192	221	413
Totals		200	176	376	514	904	1,418	714	1,080	1,794

trap," which incorporated "attractive female odor producing parts" in the adhesive to attract and trap male gypsy moths (*Porthetria dispar* (L.)). Recently Jacobson et al. (1960) isolated the gypsy moth sex attractant in pure form and synthesized a related compound that is as attractive to the male gypsy moth as the pure chemical. Holbrook et al.[2] (1960) reviewed the development of the gypsy moth trap and the use of the female sex lure to detect infestations. Roark (1933) published a list of patents relating to devices for catching insects with adhesives. Kaloostian & Yeomans (1944) developed a standard sticky-board trap primarily for the detection of the pear psylla (*Psylla pyricola* Förster) in connection with a program to eradicate this pest from the Pacific Northwest. The most important features of this trap were its medium-yellow color and the use of Deadline® as the adhesive.

Deadline proved to be the ideal adhesive because it remained tacky indefinitely under all weather conditions. Unfortunately, its manufacture was terminated about 10 years ago. A similar product, sold under the trade name of Stikem Special® or Stickem®, has been used for a

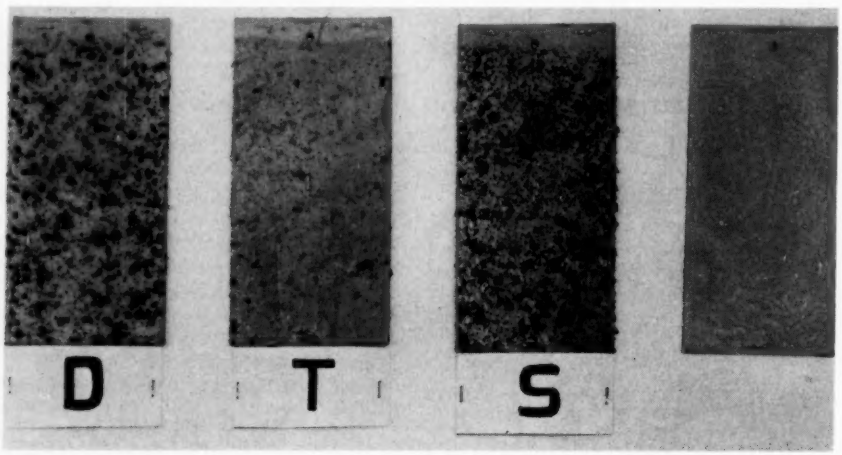

FIG. 1.—Relative effectiveness of (D) Deadline, (T) Tanglefoot, and (S) Stickem as adhesives for yellow sticky-board traps. Traps exposed in peach orchard from August 29 to November 8, 1960. Trap to far right is uncoated.

number of years as a substitute for Deadline. The relative effectiveness of Deadline, Tanglefoot, and Stickem was evaluated at Fort Valley, Ga., in 1960.

MATERIAL AND METHODS.—The disposable paper traps used in these tests were made of 5- ×10-inch binder board. The traps were sprayed with one coat of fast-drying yellow enamel paint. Yellow traffic lacquer is also satisfactory. A metal eyelet was centered about ½-inch from one end of the trap and a thin layer of Deadline, Tanglefoot, or Stickem applied on both sides. Five traps were used for each material and were hung about 5 feet above ground on branches of peach trees. The traps were exposed in one row of the orchard, one trap per tree, alternately, in the following order: Deadline, Tanglefoot, and Stickem.

Since the majority of the insects caught on the traps were Homoptera (Cicadellidae) and Diptera, only members of these orders were recorded for the purpose of this experiment. After an exposure period of 10 days, from August 29 to September 8, 1960, the insects were counted and removed from the traps. The final count was made at the termination of the exposure period on September 28. The traps shown in figure 1 were exposed from August 29 to November 8, 1960.

RESULTS AND DISCUSSION.—The results are summarized in table 1. They show the effectiveness of Deadline-coated sticky-board traps, which were used for many years in insect detection, population surveys, and collection of data in support of ecological and control studies. The data show that Stickem was equally effective and is an acceptable substitute for Deadline. Both materials were equally efficient in collecting aphids, leafhoppers, flies, small beetles, and miscellaneous insects.

Two factors contributed to the unsatisfactory results obtained with Tanglefoot as compared with Deadline and Stickem. Tanglefoot darkened the yellow traps and made them less attractive to insects that are attracted to this color. In addition the tendency of Tanglefoot to form a surface film or glaze permitted many insects to escape. Deadline and Stickem are transparent; therefore, yellow-sensitive insects are not affected by the use of these materials. These adhesives do not form surface films, do not harden at lower temperatures, and remain tacky indefinitely regardless of weather conditions.

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Control of Several Cotton Insects in Field Experiments in 1960[1]

C. B. COWAN, JR., C. R. PARENCIA, JR., and J. W. DAVIS, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.,*[2] *Waco, Texas*

ABSTRACT

Dusts or sprays of toxaphene plus DDT or Strobane® plus DDT were equally effective against the boll weevil (*Anthonomus grandis* Boheman) and the bollworm (*Heliothis zea* (Boddie)). Geigy 30494, or Bayer 29493, Shell SD-5539, and Shell SD-5533 gave fair-to-good control of the boll weevil but none of these materials were as effective as Guthion.® Shell SD-4402 plus methyl parathion gave bollworm control equal to that obtained with endrin plus DDT. Am. Cyanamid 24055 Anti-feeding Compound showed promise for control of the bollworm. Bayer 29493 and Bayer 30911 were ineffective against this pest. Methyl Trithion® plus DDT was ineffective against the cabbage looper (*Trichoplusia ni* (Hübner)) when used in a regular schedule of applications for the control of the boll weevil and bollworm. Geigy 30494, Geigy 36368, Bayer 29493, Shell SD-5533, or Shell SD-5539 were effective against the cotton leafworm (*Alabama argillacea* (Hübner)). Am. Cyanamid 24055 was also effective against this pest but kill was slow. Bayer 30911 was ineffective.

The chemical definitions of the insecticides with proprietary names mentioned above are as follows: Strobane (a mixture of chlorinated terpenes with about 66% of chlorine), Geigy 30494 (*O,O*-dimethyl *S*-(2,5-dichlorophenylmercaptomethyl phosphorodithioate), Bayer 29493 (*O,O*-dimethyl *O*-[4-methylthio)-*m*-tolyl] phosphorothioate), Shell SD-5539 (dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate), Shell SD-5533 (dimethyl 1-(*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl-phosphate), Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), Methyl Trithion (*S*-[(*p*-chlorophenylthio)methyl] *O,O*-dimethyl phosphorodithioate), Bayer 30911 (*O*-(2,4-dichlorophenyl) *O*-methyl methylphosphonothioate), Guthion (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Geigy 36368 (a mixture of Geigy 30494 and Geigy 30493 (*S*-(3,4-dichlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate) in the ratio of approximately 1:3), Am. Cyanamid Anti-feeding compound 24055 (4′-(dimethyltriazeno) acetanilide).

The effectiveness of several new insecticides for the control of certain late-season cotton insects was evaluated in field experiments conducted at Waco, Texas in 1959 (Cowan et al. 1960). In 1960 insecticides that had shown

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promise and others that later became available were included in further experiments. Although the experiments were conducted primarily for the control of the boll weevil (*Anthonomus grandis* Boheman), the bollworm (*Heliothis zea* (Boddie)), and the cotton leafworm (*Alabama argillacea* (Hübner)), information was also obtained on the control of the cabbage looper (*Trichoplusia ni* (Hübner)).

FIELD EXPERIMENTS AGAINST THE BOLL WEEVIL, BOLLWORM, AND CABBAGE LOOPER.—Five experiments were conducted with several insecticides. In experiments 1, 2, and 3, plots 16 rows wide and of sufficient length to make 0.2 acre were used. A randomized-block design with four replicates of each treatment was used for each experiment. In experiments 4 and 5, single plots 32 rows wide and 2 acres in size were used with the exception of one plot in experiment 5 that was 0.5 acre in size. The smaller plot size was used because it was treated with Am. Cyanamid Anti-feeding Compound 24055 (4′-(dimethyltriazeno) acetanilide), which was available only in limited supply.

Methods of applying insecticides were the same as those previously reported (Cowan et al. 1956). In experiments 1–5, the first two or three applications were made at 5-day intervals; 3- to 4-day intervals were used for subsequent applications.

Procedures for recording data in experiments 1–5 were the same as those reported by Ewing & Parencia (1943), and Parencia et al. (1957).

Table 1.—Effectiveness of several insecticides against the boll weevil, bollworm, and cabbage looper in field-plot experiments.

Insecticide[a]	Toxicant per Acre (lb.)	Boll Weevil Punctured Squares (%)	Per Cent Bollworm-Injured		Cabbage Loopers[b] per Plant	Seed Cotton per Acre (lb.)	
			Squares	Bolls		Total	Gain over Check
Experiment 1 (10 applications between July 5 and August 17)							
Dusts:							
Toxaphene, 20+DDT, 10	2.7 +1.35	39.2	1.0	1.2	—	1,037	719
Strobane, 20+DDT, 10	2.9 +1.45	40.2	0.9	1.2	—	989	671
Sprays:							
Toxaphene, 4+DDT, 2	2.6 +1.3	35.9	1.9	2.1	—	970	652
Strobane, 4+DDT, 2	2.6 +1.3	44.0	2.3	2.4	—	907	589
Sevin[c]	1.5	20.0	1.0	1.6	—	1,092	774
Untreated	—	69.8	8.8	9.4	—	318	—
L.S.D. at 5% level	—	19.9	1.0	1.4	—	179	—
Experiment 2 (11 spray applications between July 1 and August 24)							
Geigy 30494, 2+DDT, 2	0.5 +1.3	39.3	6.7	4.4	1.4	2,305	1,349
Bayer 29493, 4+DDT, 2	.55+1.5	29.5	13.0	9.2	1.3	2,163	1,207
Shell SD-5539, 2+DDT, 2	.43+1.3	33.8	6.1	4.8	0.8	2,345	1,389
Shell SD-5533, 2+DDT, 2	.43+1.3	35.8	7.0	4.8	.7	2,315	1,359
Guthion, 1.5+DDT, 2	.25+1.3	23.1	4.5	4.3	.7	2,446	1,490
Untreated	—	52.6	19.7	11.7	2.4	956	—
L.S.D. at 5% level	—	4.3	3.6	1.7	0.6	154	—
Experiment 3 (10 spray applications between July 8 and August 17)							
Bayer 30911, 4	1.35	77.2	7.1	8.4	0.1	771	−4
Methyl Trithion, 1+DDT, 2	0.5 +1.0	68.8	3.1	4.6	1.0	1,431	656
Methyl parathion, 2+DDT, 2	.45+1.0	68.3	3.8	3.9	0.1	1,431	656
Shell SD-4402, 1.25+Methyl parathion, 2	.5 +0.25	73.4	2.2	2.5	0.04	1,494	719
Endrin, 1.6+DDT, 2	.65+ .65	77.7	1.9	3.2	.02	1,495	720
Untreated	—	83.9	6.5	7.3	.7	775	—
L.S.D. at 5% level	—	—	2.4	2.2	.2	217	—
Experiment 4 (11 spray applications between July 1 and August 24)							
Strobane, 4+DDT, 2	2.7 +1.35	47.8	4.0	3.9	—	2,306	1,350
Toxaphene, 4+DDT, 2	2.7 +1.35	46.0	5.3	3.8	—	2,351	1,395
Guthion, 1.5+DDT, 2	0.25+1.3	23.1	4.2	3.8	—	2,182	1,226
Untreated	—	52.6	19.7	11.7	—	956	—
Experiment 5 (7 spray applications between July 26 and August 19)							
Malathion, 5+DDT, 2	1.3 +1.0	35.3	3.4	2.6	—	2,085	—
Malathion SF-60, 2.5+DDT, 2	1.3 +1.0	30.5	2.7	2.6	—	2,155	—
Am. Cyanamid 24055[d]+ Guthion, 1.5	3.0 +0.5	54.2	4.5	3.4	—	1,868	—
Toxaphene, 4+DDT, 2	2.7 +1.35	26.4	3.1	2.8	—	2,029	—

[a] Pounds per gallon in sprays; per cent active ingredient in dusts.
[b] Record on August 9 in experiment 2 and August 8 in experiment 3.
[c] Wettable powder, 85%.
[d] Wettable powder, 50%.

The results of these experiments are given in table 1. In experiment 1, each insecticide reduced the boll weevil infestation significantly below that of the check. Sevin® (1-naphthyl *N*-methylcarbamate) gave better control than a dust or spray of Strobane®(a mixture of chlorinated terpenes with about 66% of chlorine) plus DDT. No difference was shown between insecticides in bollworm control. Records of cabbage looper populations were not made but considerable ragging of leaves by this pest was observed in the plots treated with Sevin and in the untreated check plots. Each insecticide produced a significant increase in yield over the check. Sevin gave a significant increase over Strobane plus DDT spray.

In experiment 2, DDT was used for bollworm control with all applications of each insecticide except Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-totyl] phosphorothioate). Starting with the fourth application when it became evident that this material would not control the bollworm, DDT was included in all subsequent applications. Each insecticide reduced the boll weevil infestation significantly below that of the check. Guthion®(*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) gave better control than any of the other insecticides. Bayer 29493 was better than Geigy 30494 (*O,O*-dimethyl *S*-(2,5-dichlorophenylmercaptomethyl phosphorodithioate), or Shell SD-5533 (dimethyl 1-(*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate). Shell SD-5539 (dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate) was better than Geigy 30494. Each treatment reduced the bollworm infestation significantly below that of the check. Records of cabbage looper populations on August 9 showed that each insecticide reduced the infestation significantly below that of the check. Shell SD-5533 and Guthion, each with DDT, were more effective than Geigy 30494 plus DDT. Shell SD-5533 plus DDT was more effective than Bayer 29493 plus DDT. A polyhedral virus disease reduced populations and no appreciable damage occurred in the plots treated with the different insecticides. Each insecticide produced a significant increase in yield over the untreated check. Guthion plus DDT and Shell SD-5539 plus DDT gave higher yields than Bayer 29493 plus DDT. The lower yield in the latter treatment resulted from the ineffectiveness of Bayer 29493 against bollworms when this insecticide was used without DDT in the first three applications.

In experiment 3, no difference was shown in boll weevil control between any of the insecticides and the untreated check. Under an extremely heavy infestation late in July, some of the treatments gave no appreciable control and enough weevils were produced to overrun the plots treated with organophosphorus insecticides that had a short residual action even when treatment intervals were reduced to 3 and 4 days. Since the plants in this experiment produced a good bottom and middle crop before the heavy boll weevil infestation developed, damage was not so severe as the infestation records might indicate especially in treatments that gave good bollworm control. Each treatment except Bayer 30911 (*O*-(2,4-dichlorophenyl) *O*-methyl methylphosphonothioate) reduced the bollworm infestation significantly below that of the check. Each treatment except Methyl Trithion (*S*-[(*p*-chlorophenylthio)methyl] *O,O*-dimethyl phosphorodithioate) reduced the cabbage looper infestation below that of the check. Endrin plus DDT and Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) plus methyl parathion were particularly effective. Each insecticide except Bayer 30911 produced a significant increase in yield over that of the untreated check. The low yield in the plots treated with Bayer 30911 was because of lack of bollworm control.

In experiment 4, Guthion plus DDT gave better boll weevil control than Strobane or toxaphene plus DDT. No difference in bollworm control was shown between insecticides. Good increases in yield were produced by all of the insecticides.

In experiment 5, malathion SF-60 was slightly more effective against the boll weevil than the same dosage of regular malathion. Toxaphene plus DDT gave better control than either of the malathion treatments. Results of an experiment conducted earlier in the season had shown that Am. Cyanamid Anti-feeding Compound 24055 afforded little boll weevil control; therefore Guthion was added to this material. The plot treated with this combination material was situated on the edge of the field and subjected to heavy migration pressure; thus the boll weevil infestation could not be reduced to a satisfactory level. Slightly less bollworm egg deposition occurred in the plot treated with Am. Cyanamid 24055 plus Guthion than in the other treated plots. No appreciable difference was shown in injured squares and bolls between treatments.

FIELD EXPERIMENTS AGAINST THE COTTON LEAFWORM.—Two experiments were conducted in single plots 16 rows wide and 1 acre in size. One spray application of the insecticides was made on August 17 in the first and on September 8 in the second experiment. Immediately after application 1-pint ice-cream-carton cages were placed over foliage on which larvae were found and mortalities were recorded at various intervals after application. Fifteen leafworms were caged in each plot.

In experiment 1, 0.25-pound-per-acre dosages of methyl parathion, Geigy 30494, Bayer 29493, Shell SD-5539, and Shell SD-5533 each gave 100% kill of larvae 1 day after treatment. Am. Cyanamid 24055 gave a kill of 53% after 1 day and 67% after 2 days. Kill was also determined by counting all larvae, before and 1 and 2 days after treatment, on 15 plants selected at random and tagged for observation in the plots receiving the different insecticides. Kills recorded by this method were quite similar to those obtained in the cages.

Observations of the plots on September 8, 22 days after treatment, showed that methyl parathion had afforded considerable residual control of the next generation of cotton leafworm larvae.

In the second experiment (table 2), toxaphene-DDT, Strobane-DDT, Shell SD-4402, Geigy 30494 and Geigy 36368 (a 1:3 mixture of Geigy 30494 and Geigy 30493 (*S*-(3,4-dichlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate) gave high kills by the end of the second day. Little evidence of larval feeding was seen throughout a 6-day period during which observations were made in the cages where the Am. Cyanamid 24055 treatment was used. The check cages had to be moved several times because of complete defoliation by larval feeding. Further evidence of very limited feeding was obtained in the plot treated with Am. Cyanamid 24055 in wire-screen cages measuring 2 feet on a side placed over

Table 2.—Effectiveness of several insecticides against the cotton leafworm.

Insecticide (lb./gal.)	Toxicant per Acre (lb.)	Net Per Cent Kill at Indicated Days After Treatment				
		1	2	4	5	6
Toxaphene, 4+DDT, 2	2.0+1.0	73.3	100	—	—	—
Strobane, 4+DDT, 2	2.0+1.0	80.0	100	—	—	—
Shell SD-4402, 1.25	0.5	93.3	92.2	91.7	—	—
Geigy 30493, 2	.25	60.0	53.9	58.4	—	—
Geigy 30494, 2	.25	86.7	92.2	91.7	—	—
Geigy 30368, 2	.25	80.0	92.2	91.7	—	—
Bayer 30911, 4	.5	20.0	38.5	33.4	—	—
Am. Cyanamid 24055[a]	3.0	33.3	38.5	66.7	83.4	83.4
Untreated	—	13.3	20.0	—	—	—

[a] Wettable powder, 50%.

each of the plants. Kills in these cages were 11%, 17%, 70%, 86%, and 90% after 1, 2, 4, 5, and 6 days, respectively. Fifty dead larvae were found 4 days after treatment in an area 6 feet long between two cotton rows in this plot.

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Experiments with Concentrated DDT Sprays for European Pine Shoot Moth Suppression in Forest Plantations[1]

William E. Miller and Dean L. Haynes,[2] *Lake States Forest Experiment Station, Forest Service, U.S.D.A., East Lansing, Michigan*

Abstract

Dosage, volume, and timing experiments with concentrated DDT sprays were carried out against the European pine shoot moth (*Rhyacionia buoliana* (Schiffermüller)) in red pine (*Pinus resinosa* Ait.) plantations in Michigan from 1956 to 1958. Concentrated sprays were applied by several types of mistblowers, as well as by fixed-wing aircraft. Simulated concentrated sprays were applied by backpack sprayers. The least DDT dosage in mistblower treatments consistently giving 90% or greater population reduction was 10 pounds of actual toxicant per acre; the least volume, 40 gallons of water spray per acre. Under some conditions, less DDT dosage and volume seemed to suffice. Oil sprays in summer at rates of 10 or more gallons per acre caused foliage browning. The period available for applying concentrated sprays to achieve 90% or greater reduction in population in the spring is 2 weeks or less, depending on dosage, in contrast to the 4 weeks available for applying diluted sprays.

From 1956 through 1958, the writers carried out field investigations with concentrated DDT sprays for control of the European pine shoot moth (*Rhyacionia buoliana* (Schiffermüller)) in forest plantations of red pine (*Pinus resinosa* Ait.) in Lower Michigan. The effectiveness of DDT against this insect had previously been demonstrated in the field by means of diluted sprays applied to individual trees (Stearns 1953, Miller & Neiswander 1955, and Morofsky et al. 1956). Similar results have been obtained in later experiments (Guyer et al. 1957, Butcher & Haynes 1959, Donley 1960).

Diluted sprays require more spray per acre than is normally practical for forest plantations. The work reported here was done to explore dosages, volumes, and timing of concentrated sprays applied on an area basis rather than to individual trees. The DDT, 25%, used was in the form of emulsifiable concentrate. All treatments were assigned at random to plots in plantings where trees were spaced at 6×6 feet and were between 4 and 6 feet tall. Analysis of variance with a multiple range test (Duncan 1955) was used in the statistical evaluations.

Dosage-Volume Experiments.—The three series of experiments described below led to the conclusion that concentrated sprays applied with ground equipment using the airblast principle can be as effective as diluted sprays if appropriate concentrations are used and adequate coverage is secured. It is also evident that such sprays can be equally effective against spring and summer larvae. Based on these investigations, suggested dosages and rates of DDT for spraying with a large mistblower were formulated (Miller & Haynes 1958a).

First Series.—DDT at various dosages up to 4 pounds of actual toxicant in 20 gallons of No. 2 fuel oil per acre was applied as airblast and airplane treatments in the spring and summer of 1956. These treatments were timed to coincide with the beginning of external larval activity (spring) or hatching (summer).

Essentially the same experiment was installed in two different plantations each season. One-acre plots with dimensions of 1×10 chains (66×660 feet) were used except in the fogging test where a $\frac{1}{4}$-acre plot was used.

Airplane sprays were applied in the spring at the rate of 5 and 10 gallons per acre, with 1 pound of DDT per 5 gallons of oil. The 5-gallon-per-acre applications were secured in one pass, while the 10-gallon rate was secured

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[2] Dr. Miller works at the Station's East Lansing, Mich., field office, which is maintained in cooperation with Michigan State University. Mr. Haynes, who worked at the same office at the time of the studies reported here, is now at the Forest Biology Laboratory, Fredericton, New Brunswick.

in two passes made in opposite directions. Different equipment used in the summer necessitated two and four passes, respectively, to achieve the same application rates as in the spring. Oil-sensitive cards placed an inch or two above ground level between the trees on each plot suggested that about 10% of airplane output reached the trees.

The airblast machine used (Lawrence Aero-mist Model L-40) generated an airblast estimated at 2,000 cubic feet per minute and had a spray pump capacity of 12 gallons per minute. The 10-row plots were sprayed from both sides. The amount of mist each plot received was regulated by varying the speed at which the machine moved.

Treatments were evaluated about 7 weeks after application by comparing sprayed populations with those in untreated plots. The sample consisted of 10 trees systematically chosen from the middle two rows of each plot and distributed along the full length of plots. For the spring treatments, the actual number of living and parasitized insects was counted on each sample tree. In the summer, however, the number of apparently infested bud clusters per tree was counted. This method, rather than that of counting the number of actual insects, is much faster, and has subsequently been shown to be adequate where only one insecticide is under test (Butcher & Haynes 1959).

Airblast applications gave reductions ranging from 14% to 80% (table 1). With increasing amounts of toxicant and total spray volume, reductions increased. In summer, however, oil treatments of 10 or more gallons per acre caused foliage browning, but no tree mortality. The spring airblast treatments were applied 10 days after first external larval activity was observed; in a later section of this paper it will be shown that this date was probably too late for best results.

Airplane treatments gave reductions ranging from 3% to 28% (table 1). The fogging treatment (table 1) gave erratic results, with much variation occurring within the plot. Trees very close to the generator received coarse spray droplets which resulted in a whitish residue, persisting for weeks, and which produced high kills, but trees merely enveloped in the fog showed little if any larval control.

Table 1.—Results of concentrated DDT applications made in spring and summer, Ottawa County, Mich., 1956.[a]

Means of Application	Mixture per Acre		Mean Number Insects or Injured Tips per Tree After Treatment		Composite Percentage Reduction from Check
	Lb. DDT	Gal. Oil			
			Spring control tests		
			Plantation 1	*Plantation 2*	
Airplane	1	5	28	19	4
	2	10	26	9*	28
Mistblower	1	5	27	15	14
	2	10	17*	9*	47
Check			36	13	
			Summer control tests		
			Plantation 3	*Plantation 4*	
Airplane	1	5	137	41*	7
	2	10	129	57	3
Mistblower	1	5	83*	33*	39
	2	10	63*	21*	56
	4	20		12*	80
Fogger[b]	10	5	97		27
Check			132	59	

[a] Spring airblast treatments applied May 3, 19 days after incipient larval activity (+10); spring airplane treatments, April 25 (+2). Summer treatments applied over a 5-day period beginning June 28 (−7 to −2).
[b] A hand-carried, pulse-jet fog maker (Swingfog).
* Indicates significant reduction from check at 5% level.

Second Series.—The unsatisfactory reductions obtained in the first series of tests prompted the use of higher concentrations of DDT and higher total spray volumes. Oil sprays were also abandoned in favor of water sprays because of foliage browning.

In contrast to the 4 pounds of DDT and less per acre used in the first series of mist and aerial sprays, the second series were applied at rates of 10 to 30 pounds of actual DDT in 10 to 40 gallons of water per acre. This amount of the toxicant is more nearly similar to that applied in diluted sprays. From diluted spray tests with 2 pounds of DDT per 100 gallons of spray, carried out by the authors and others, it was determined that the per-acre application rate of this type of spray amounted to several hundred gallons. A rule of thumb was developed for estimating diluted spray quantities needed per acre: When applied to the point of runoff with power equipment in plantations with about 1,000 trees to the acre (6- ×6-foot spacing), 100 gallons of spray will be needed per foot of tree height per acre. For example, spraying an acre of 5-foot trees would require about 500 gallons of spray per acre; at 2 pounds of actual DDT per 100 gallons of spray, the DDT rate per acre would be 10 pounds.

The second series of tests was directed against the summer larvae. An airblast machine (John Bean 100-TE Rotomist), with an air movement capacity of 28,000 cubic feet of air per minute and a spray pump capacity of 7 gallons per minute, was used. All treatments were dispersed from both sides of the 10-row plots. The spray had sufficient velocity to pass completely through plots so that each tree was treated on two sides.

The treatments were evaluated 8 weeks after they were applied. Twenty sample trees were systematically taken in each plot and the number of apparently infested tips on the top three whorls of each sample tree was counted.

In this series, 92 or more per cent reductions were obtained with 10 pounds of DDT per acre in spray volumes of 40 gallons in Plantation 1 and 20 or more gallons in Plantation 2 (table 2). Reductions decreased with diminishing spray volumes, but at different rates in the two plantations. For example, in Plantation 2, 20 gallons of spray per acre reduced the population as much as 40 gallons did in Plantation 1. Also, 10 gallons of spray gave less than half the reduction in Plantation 1 than it did in Plantation 2. This result probably reflects a difference in density of foliage between the two plantings. The dense, bushy foliage of Plantation 1 was the result of a long history of shoot moth infestation. This plantation had a mean of 63 shoots in the upper three whorls with a 95% confidence interval of 60 to 66. Plantation 2 had a

Table 2.—Results of DDT airblast applications made with a medium-sized mistblower in summer, Ottawa County, Mich., 1957.[a]

Mixture per Acre		Plantation 1 (dense foliage)[b]			Plantation 2 (sparse foliage)[b]		
Water (gal.)	Actual DDT (lb.)	Mean Number Shoots Infested per Tree After Treatment	Percentage Reduction from Check	Significant Difference[c]	Mean Number Shoots Infested per Tree After Treatment	Percentage Reduction from Check	Significant Difference[c]
40	30	0.4	99	I	0.8	97	I
40	20	.6	99	I	.2	99	I
40	10	3.1	93	I	2.1	92	I
20	10	10.5	75	II	2.1	92	I
10	10	28.1	34	III	5.2	80	II
Check		42.7		IV	25.4		III

[a] Application dates were June 25 to July 1, 5 days before to 1 day after incipient hatching.
[b] Plantations 1 and 2 had means of 63 and 35 shoots on the upper three whorls per tree. These means were significantly different at the 1% level.
[c] Any two means with the same numeral are not significantly different at the 5% level.

mean of 35 shoots, and a 95% confidence interval of 32 to 38. Logically, dense foliage would require more spray for the same coverage than sparse foliage, and dense foliage would also create more barrier effect to a spray blast than sparse foliage.

This differential performance of the sprays in plantations of different foliage density led to a dual recommendation (Miller & Haynes 1958a): 7 pounds of DDT in 35 gallons of spray per acre if trees are less than 4 feet tall, and 10 pounds in 50 gallons if trees are more than 4 feet tall.

Third Series.—The satisfactory reductions in population secured in the summer with concentrated sprays in 1957 led to similar tests against larvae resuming activity in the spring. In this series, two application rates, consisting of 5 pounds of DDT in 20 gallons of water and 10 pounds in 40 gallons of water per acre, were used. The mistblower had to be moved around the ½-acre 1- ×5-chain plots from 2 to 4 times at the slowest tractor speeds to attain the desired application rates. Posttreatment insect counts were made 6 weeks after the application. The number of insects was counted on 20 systematically chosen sample trees per plot.

The 5-pound, 20-gallon treatment resulted in 73% reduction and the 10-pound, 40-gallon in 91% (table 3). Insofar as they can be compared, these results were similar to those of summer tests with this machine.

The possibility that a larger airblast machine might do in one pass what it took the above machine several passes to do was tested in the summer by the Lower Michigan National Forest. A mistblower designed for row-crop work (John Bean 15 RD Aircrop) (illustrated by Miller & Haynes 1958a) was used in a shoot moth suppression operation to apply 10 pounds of DDT in 50 gallons of water per acre. This machine generated an airblast of 31,000 cubic feet per minute and had a pump capacity of 35 gallons per minute. About 12 rows of trees were sprayed at a time from each side, and there was enough carry-through spray so that all trees were treated from both sides. The two areas treated were so large that only infested and uninfested trees were recorded in the control evaluation 8 weeks after treatment. Adjacent untreated parts of the same areas were used as checks. Of 50 trees examined in each sprayed plantation (taking every 10th tree in every 10th row), 5% and 64% were infested, whereas 100% were infested in the untreated parts. The poorer control in the second plantation is attributed to excessive hardwood brush which had obstructed the airblast and shielded many trees.

Table 3.—Results of DDT applications made in spring with a medium-sized mistblower in Ottawa County, Mich., 1958.[a]

Actual DDT (lb.)	Water (gal.)	Mean Number Insects per Tree After Treatment	Percentage Reduction from Check
5	20	2.4	73
10	40	.8	91
Check		8.9	

[a] Application date was April 18, 1 day before incipient larval activity.

Spray Timing Experiments in the Spring.—Simultaneous "moist" and "drench" sprays were applied at intervals of about 10 days during early spring with backpack sprayers equipped with cone-type nozzles. The moist sprays were intended to simulate concentrated DDT sprays and went on at an average rate of 50 gallons per acre (assuming 1,000 trees to the acre). The drench or diluted sprays were included for comparative purposes and were applied at an average rate of 110 gallons per acre. The toxicant in both sprays consisted of DDT at rates of 2, 4, and 8 pounds per 100 gallons of water. The treatments were applied to single trees and replicated 25 times for each spray date. Complete experiments consisting of both moist and drench treatments were installed in two different locations in 1957. The drench experiment was continued in 1958, likewise in two locations.

Treatments were evaluated about 4 weeks after the last spray was applied. The number of currently infested tips per tree was counted. Results of all drench or diluted spray treatments were combined for graphical presentation in figure 1, and those of all simulated concentrated treatments were combined for presentation in figure 2. The point of reference for meaningfully combining these data was the relationship of treatment dates to local date of incipient larval activity.

Nearly all the diluted sprays applied within about 2 weeks before or 2 weeks after initial spring larval activity gave 95% or more reduction (fig. 1). The population

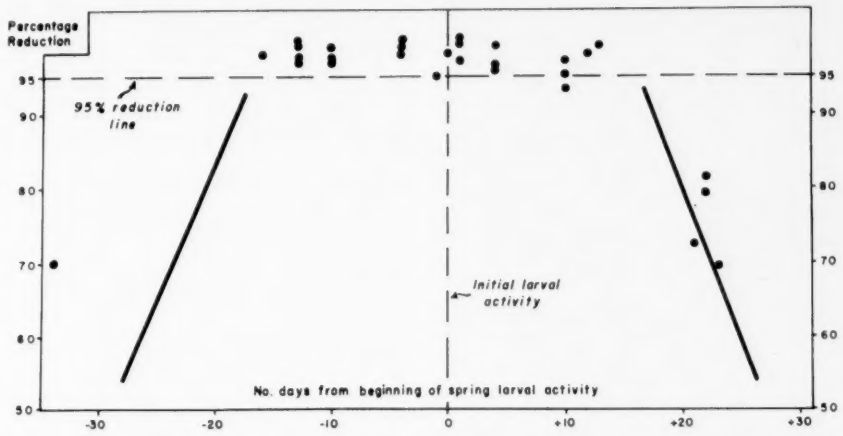

FIG. 1.—Composite results of DDT "drench" sprays applied at intervals in the spring. The trend lines were sight fitted on the basis of the points shown as well as several points below 50% reduction which are not shown.

means of such reductions ranged from 0 to 1.0 infested tips per tree. Treatments applied earlier or later than this month-long optimum period gave markedly poorer results. There was no difference in performance between dosages of 2, 4, or 8 pounds of DDT per 100 gallons of spray.

With the simulated concentrated sprays, there was a much shorter optimum application period. Shoot moth reductions of 95% or better were indicated on the diagram over a period ranging roughly from a few days to a week depending on DDT dosage (fig. 2). A few more days were indicated for reductions of 90 per cent upward. Most of this period occurred after incipient larval activity. Population means of the 95% or greater reductions ranged from 0.1 to 0.4 infested tips per tree.

The most concentrated spray of this experiment (8 pounds DDT per 100 gallons) had been applied at the rate of about 4 pounds in 50 gallons per acre. This was less than half the toxicant rate used in the successful airblast treatments. The success of the moist sprays at lower DDT dosages is attributed to better coverage. The coverage achieved with the hand-pointed nozzles of the backpack sprayers was doubtless better than with the mechanical spray dispensing of the mist machines.

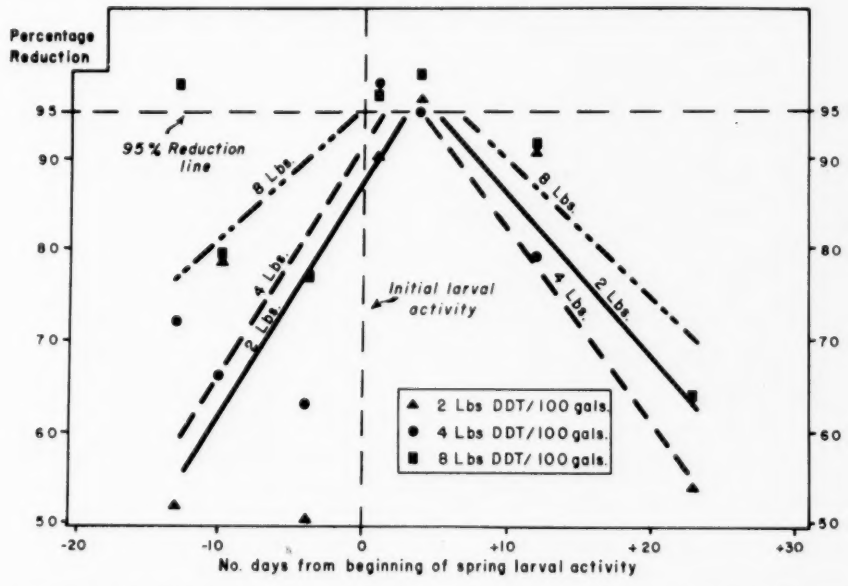

FIG. 2.—Composite results of DDT "moist" sprays applied at intervals in the spring. The trend lines were sight fitted on the basis of the points shown as well as 1 point below 50% reduction which is not shown.

The results of the simulated concentrated sprays help to explain the poor performance of the 1956 spring airblast treatments discussed earlier. A very narrow latitude for the timing of concentrated sprays also suggests an explanation for the erratic results experienced in the past with mistblower applications of DDT against the shoot moth.

With diluted sprays, spring scheduling can probably be done accurately enough by calendar date (Miller & Haynes 1958b). With concentrated sprays, calendar date can also be used as a means for setting a tentative spray date, but first external larval activity (first appearance of resinous tents) is most reliable for indicating actually when to start spraying.

The period available for applying diluted DDT sprays against the summer larvae (Miller & Neiswander 1955) is similar to that for spring larvae, but it may be a little shorter. Therefore, the same procedure for scheduling diluted sprays in the spring would seem to be adequate also in the summer, that is, by calendar date (Miller & Neiswander 1955). With concentrated sprays, calendar date might best serve as a guide in the summer as in the spring, with main dependence being placed on hatching (first appearance of resinous tents) for actually starting spray operations.

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Resistance to the Mexican Bean Beetle in Several Bean Genera and Species[1]

DAN WOLFENBARGER and J. P. SLEESMAN,[2,3]
Ohio Agricultural Experiment Station, Wooster

ABSTRACT

Nine *Phaseolus* species, the cowpea (*Vigna sinensis* (Torner) Savi), the broad bean (*Vicia faba* L.), the soybean (*Glycine max* (L.) Merrill), and the Bonavist bean (*Dolichos lablab* L.) varied in resistance to the Mexican bean beetle (*Epilachna varivestis* Muls.). *Phaseolus aureus* Roxb., *P. mungo* L., *P. calcaratus* Roxb., *P. radiatus* L., *P. atropurpureus* DC., *P. lathyroides* L., *P. polystachyus* L., *Glycine max*, *Vicia faba*, *Dolichos lablab*, and *Vigna sinensis* lines exhibited varying degrees of resistance to beetle-feeding damage under field conditions. All *Phaseolus vulgaris* L. lines, one *Phaseolus acutifolius* A. Gray line, and the progenies of interspecific *Phaseolus* crosses were susceptible. *Phaseolus lunatus* lines ranged from intermediate in resistance to susceptible.

Two methods, larval counts and a visual rating of leaf feeding damage, were compared in evaluating the various lines for resistance to the bean beetle. The visual rating method was found to be as satisfactory as larval counts.

Mean maturity dates were associated with resistance in *Phaseolus* spp., the fewer days to maturity the lower the leaf-feeding resistance exhibited.

The common bean (*Phaseolus vulgaris* L.) is frequently defoliated by the Mexican bean beetle (*Epilachna varivestis* Muls.). The purpose of this investigation was to identify resistance in *Phaseolus* and related genera and compare two methods of evaluation.

List (1921), Crawford (1924), and Eddy & McAllister (1927) stated that the Mexican bean beetle freely attacks the common and lima bean types. List (1921) reported that some differences in susceptibility did exist in varieties of the common bean varying from 20% to 95% in leaf feeding damage. He also stated that the best crops were produced by the early maturing varieties and in most cases these varieties showed the greater amount of injury. Thomas (1924) and Howard & English (1924) stated that *P. vulgaris* varieties are preferred by the Mexican bean beetle to the lima bean. Howard (1930) reported that the beetle has a decided preference for the pinto, navy, lima,

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[2] Research Assistant and Professor, respectively.

[3] Seed used in these investigations were supplied by R. E. Wester, U. S. Department of Agriculture, Crops Res. Div., Beltsville, Md.; A. B. Strand, Dandridge, Tennessee; L. W. Hudson, Regional Plant Introduction Sta., Pullman, Washington; A. P. Lorz, Dept. Vegetable Crops, Fla. Agric. Expt. Sta., Gainesville, Florida; D. D. Dolan, Plant Introduction Sta., N. Y. Agric. Expt. Sta., Geneva, New York; Asgrow Seed Co.; Rogers Bros. Seed Co.; Northrup, King & Co.; and Ferry-Morse Seed Co.

kidney and snap beans. Friend & Turner (1931) stated that the common, runner, and lima bean are the preferred hosts of the bean beetle. During 1955–57, Campbell (1958) reported that Triumph, Baby Fordhook, Wade, Evergreen, and Idaho Refugee were resistant and that they showed 24.8%, 25.2%, 28.9%, 29.5%, and 31.3% foliage injury, respectively.

List (1921) reported that the broad bean (*Vicia faba* L.) is free of attack by the bean beetle. Thomas (1924), Crawford (1924), Howard (1924), and Eddy & McAllister (1927) reported that rarely have soybeans (*Glycine max* (L.) Merrill) been seriously attacked, whereas Strand (1943) stated that soybeans were practically defoliated in Tennessee.

Friend & Turner (1931) stated that the soybean, cowpea (*Vigna sinensis* (Torner) Savi), and Bonavist bean (*D. lablab* L.), although damaged, were not preferred hosts. Howard (1924), Howard & English (1924), and Friend & Turner (1931) found that the mungo bean (*Phaseolus mungo* L.) and the mung bean (*Phaseolus aureus* Roxb.) were practically immune from injury and are rarely fed upon. Friend & Turner (1931) reported that the mung bean and broad bean are free of or immune to attack. Strand (1943) stated that the mungo bean is highly resistant to bean beetle feeding, whereas the Adzuki (*Phaseolus angularis* Willd.) and common bean are about equal in susceptibility. He reported also that certain lines of an interspecific cross between the susceptible Asgrow Stringless Greenpod (*P. vulgaris*) and the resistant mungo bean possess more resistance than others to bean beetle feeding.

MATERIALS AND METHODS.—At Marietta, Ohio, in 1958, 1959, and 1960, 20 to 40 seeds of various bean genera and species were planted in single 5-foot rows separated by a 3-foot space between the rows and between the plots in the row.

In 1958, larval counts and a nine-class leaf feeding damage rating were made, whereas during 1959–60 only the leaf feeding damage ratings were recorded. The visual ratings used for classifying the leaf surface area damaged were as follows:

Class 1 = no feeding;	
Class 2 = 1 to 9%	Class 6 = 40 to 49%
Class 3 = 10 to 19%	Class 7 = 50 to 69%
Class 4 = 20 to 29%	Class 8 = 70 to 89%
Class 5 = 30 to 39%	Class 9 = 90 to 100%

In the relative leaf feeding rating scale, lines which rated 1 to 3 were considered resistant, lines which rated 4 to 6 were considered intermediate in resistance, and lines which rated 7 to 9 were considered highly susceptible.

Figure 1 shows characteristic damage in classes 1, 4, and 9, respectively, of the described visual leaf damage ratings.

RESULTS AND DISCUSSION.—The data obtained from field evaluations show in table 1 that *Phaseolus aureus*, *P. mungo*, *Phaseolus calcaratus* Roxb., *Phaseolus radiatus* L., *Phaseolus atropurpureus* DC., *Phaseolus lathyroides* L. Selection 22, *Glycine max*, and *Vicia faba* were free of leaf feeding damage. *Dolichos lablab*, *Vigna sinensis*, and *Phaseolus polystachyus* L. exhibited a lower degree of resistance. *Phaseolus lunatus* L. lines ranged from intermediate in susceptibility to susceptible. *P. lunatus* PI 194314 was the most resistant. Wester (1959) reported that at Beltsville, Md., *P. lunatus* 4913 was the most resistant and that Piloy, Thaxter, and Fordhook Bush

FIG. 1.—Classes of relative leaf feeding damage by the Mexican bean beetle (Top) Class 1 shows no injury (*P. calcaratus*); (Middle) Class 4 shows intermediate injury (*P. lunatus* PI 194314); (Bottom) Class 9 shows severe injury (*P. vulgaris* × *P. mungo* No. 12 throwback).

Table 1.—Reaction of several genera and species of beans to the Mexican bean beetle. Marietta, Ohio. 1958–60.

Species	Variety, Accession No., or Strain	Class of Leaf Feeding Damage[a]				
		Midseason Evaluation	Final Evaluation			
		1960	1958	1959	1960	Mean Final Evaluation
		July 20	August 4–5	July 21	August 12	
P. aureus	PI 164889	1.0		1.0	1.0	1.0
P. aureus	PI 207504	1.0		1.0	1.0	1.0
P. lathyroides	Selection 22		1.0			1.0
P. mungo	PI 101581	1.0	1.0	1.0	1.0	1.0
P. calcaratus		1.0		1.0	1.0	1.0
P. atropurpureus		1.0			1.0	1.0
P. radiatus	57703	1.0			1.0	1.0
P. radiatus	57704			1.0		1.0
V. faba	Broad Windsor	1.0			1.0	1.0
V. faba	Best of All Broad Bean	1.0			1.0	1.0
G. max	Chippewa	1.0		1.0	1.0	1.0
G. max	Blackhawk	1.0		1.0	1.0	1.0
G. max	Harosoy	1.0		1.0	1.0	1.0
V. sinensis	73-05110	1.0		1.0	1.0	1.0
V. sinensis	18-17-2110	1.0		1.0	1.0	1.0
V. sinensis	644210	1.0		1.0	2.0	1.5
V. sinensis		1.0	1.0	2.0	2.0	1.8
D. lablab	Bonavist Bean	1.0			2.0	2.0
P. polystachyus				3.0		3.0
P. lunatus	PI 194314	1.0		2.5	4.0	3.3
P. lunatus	PI 180462	3.0		3.5	5.0	4.3
P. lunatus	PI 183412	2.0		4.0	5.0	4.5
P. lunatus	Early Thorogreen	1.0		3.0	6.0	4.5
P. lunatus	Fordhook C2	1.0		3.0	6.0	4.5
P. lunatus	PI 195344	1.0		4.0	5.0	4.5
P. lunatus	PI 195339	1.0		3.0	7.0	5.0
P. lunatus	PI 195340	2.0		3.0	7.0	5.0
P. lunatus	PI 164891	1.0		4.0	6.0	5.0
P. lunatus	Burpee's Improved Bush	1.0		2.5	8.0	5.2
P. lunatus	PI 162668	1.0		3.5	7.0	5.3
P. lunatus	Fordhook 242	1.0		4.0	7.0	5.5
P. lunatus	PI 162689	1.0		4.0	7.0	5.5
P. lunatus	PI 180324	3.0	4.0		7.0	5.5
P. lunatus	Thorogreen	2.0		2.0	8.0	6.0
P. lunatus	PI 164893	3.0		4.0	8.0	6.0
P. vulgaris	PI 181786		5.5	7.0		6.3
P. vulgaris	PI 169903		6.0	7.0		6.5
P. lunatus	PI 209051	1.0		6.5	7.0	6.8
P. lunatus	Piloy	2.0			7.0	7.0
P. lunatus	4913	1.0			7.0	7.0
P. lunatus	Limagreen	2.0			7.0	7.0
P. vulgaris	PI 172025	2.0	6.0	7.0	8.0	7.0
Wade×*P. lathyroides*				7.0		7.0
P. vulgaris	PI 177514	2.0	6.5	8.0	8.0	7.5
P. vulgaris	PI 169794	3.0	6.5	8.0	9.0	7.8
P. vulgaris	U. S. Refugee #5	1.0	7.5	8.0		7.8
P. vulgaris	White Half Runner	4.0		7.0	9.0	8.0
P. vulgaris×*P. lathyroides*	Selection 22		7.5	8.5		8.0
P. lunatus	Wood's Prolific	7.0			8.0	8.0
P. lunatus	Thaxter	1.0			8.0	8.0
P. lunatus	Dixie Butterpea	3.0			8.0	8.0
P. vulgaris	Stringless Red Valentine	8.0	8.0	9.0		8.5
P. vulgaris	Bountiful Canner	1.0	8.0	9.0		8.5
P. vulgaris	White Seeded Refugee	1.0	9.0	8.0		8.5
P. vulgaris	Idaho Refugee	1.0	8.0	8.5	9.0	8.5
P. vulgaris	Wade	2.5	7.5	9.0	9.0	8.5
P. vulgaris	Sweetheart	5.0	8.0	9.0		8.5
P. vulgaris	Improved Supergreen	1.0	8.3	9.0		8.7
P. vulgaris	Topmost	1.0	8.6	9.0		8.8
P. vulgaris	Tennessee Greenpod	4.0	8.3	9.0		8.8
P. vulgaris	Plentiful	4.0	8.7	9.0		8.9
P. vulgaris	Dwarf Horticultural	3.0	9.0	9.0		9.0
P. vulgaris	Tenderlong-15	4.0	9.0	9.0	9.0	9.0

[a] Based on 1 least, 9 most.

Table 1.—(*Continued*)

Species	Variety, Accession No., or Strain	Class of Leaf Feeding Damage[a]				
		Midseason Evaluation	Final Evaluation			Mean Final Evaluation
		1960	1958	1959	1960	
		July 20	August 4–5	July 21	August 12	
P. vulgaris	Sulfer	7.0			9.0	9.0
P. vulgaris	Blue Ribbon	4.0			9.0	9.0
P. vulgaris	Gardengreen	1.0	9.0	9.0		9.0
P. vulgaris	Genuine Cornfield	4.0			9.0	9.0
P. vulgaris	Italian or Romano	4.0			9.0	9.0
P. vulgaris	Morses Pole 191 (U. S. #4)	4.0			9.0	9.0
P. vulgaris	Selected Canadian Wonder	5.0			9.0	9.0
P. vulgaris	Asgrow Black Valentine	3.0	9.0	9.0		9.0
P. vulgaris	Ranger	5.0	9.0	9.0		9.0
P. vulgaris	Topcrop	2.0	9.0	9.0		9.0
P. vulgaris	Cooper Wax	2.0	9.0	9.0		9.0
P. lunatus	Small White Lima	2.0			9.0	9.0
P. lunatus	King of the Garden	3.0			9.0	9.0
P. lunatus	Clark's Bush	1.0			9.0	9.0
P. vulgaris×*P. mungo*	No. 12 throwback	3.0			9.0	9.0
P. vulgaris×*P. mungo*	No. 12 segregating	3.0			9.0	9.0
P. acutifolius	PI 200920	5.0		9.0	9.0	9.0

follow in an increasing order of susceptibility, whereas at Marietta these lines were all susceptible.

Progenies of crosses between resistant and susceptible *Phaseolus* (from A. B. Strand) species were susceptible. No resistant segregates were noted. Data in table 1 support the report by Strand (1943) that resistance appears to be a recessive characteristic.

Almost all *P. vulgaris* lines exhibited susceptibility to beetle feeding damage. Only two *P. vulgaris* Plant Introductions (PI 181786 and PI 169903) exhibited an intermediate degree of resistance in the final evaluation. Strand (1959) and Campbell (1958) stated that Wade was the most resistant of *P. vulgaris* varieties; however, the data presented in table 1 do not support this conclusion. Strand (1959) stated that Wade, with its dark green foliage, showed more resistance than varieties with light colored foliage such as Refugee, although Campbell (1958) reported that Idaho Refugee was almost as resistant as Wade. One *P. acutifolius* A. Gray line was highly susceptible.

Table 2.—Mean leaf-feeding damage to *Phaseolus* spp., interspecific *Phaseolus* crosses, *V. faba*, and *G. max* by the Mexican bean beetle in relation to maturity date and evaluation date. Marietta, Ohio. 1958–60.

Species	Number Lines Matured	Mean Maturity Date[a]	Leaf-Feeding Damage[b] 1960	Mean Leaf-Feeding Damage[b]	Number Lines Evaluated
P. aureus	4	124	1.0	1.0	7
P. mungo	1	116	1.0	1.0	1
V. faba	2	100	1.0	1.0	2
P. radiatus	1	123	1.0	1.0	2
G. max	6	117	1.0	1.0	6
P. polystachyus	1	98	—	3.0	1
P. lunatus	14	110	1.9	5.5	29
P. vulgaris× *P. lathyroides*	1	104	—	8.0	1
P. vulgaris	63	94	2.9	8.5	63
P. vulgaris× *P. mungo* #12	2	97	3.0	9.0	2

[a] Class of Leaf Feeding Damage: 1 = 0% defoliation; 3 = 10% to 30% defoliation; 5 = 40% to 60% defoliation; 7 = 70% to 80% defoliation; 9 = 90% to 100% defoliation.

[a] 90% pods dry as exhibited on each plot.

[b] Based on 1 least, 9 most; 27 days to midseason evaluation in 1960. Days to final evaluation: 76 in 1958; 62 in 1959; 80 in 1960.

In 1958, July 12 larval counts vs. the August 5 classes of visual ratings were compared by a simple correlation on 1456 susceptible *P. vulgaris* lines. The correlation coefficient ($r = +0.86$ at 1% level) indicated a close linear relationship between mean larvae and the leaf feeding rating. However, lines that exhibited a complete defoliation (August 5 visual rating) might have had no larvae present when the midseason or July 12 count was made. Of the lines in Class 9 (90% to 100% leaf surface area damaged per plot), 7.2% had zero larvae on July 12 and yet at the end of the season the plots were defoliated. Since leaf feeding damage ratings differentiate resistant from susceptible lines with less effort and time than counting larvae it is a better method to use.

Among *Phaseolus* species listed in table 2, a correlation coefficient ($r = -0.90$ at 1% level) was found between maturity and mean leaf feeding damage indicating that resistance is found in *Phaseolus* species which take longer to mature. In the midseason evaluation, *P. vulgaris* lines were fed upon approximately 100% more than *P. lunatus* lines. Final evaluation showed an 80% increase in leaf feeding which indicates that lima beans were more tolerant than the common bean.

The various species exhibited a 30-day differential (ranging from 94 to 124 days) in maturity. Data in table 2 show that final evaluations of plots in the 1958–60 seasons were made in 76, 62, and 80 days after planting, respectively. Many of the *P. vulgaris* lines had already matured (80 days after planting) whereas 62 days did not allow sufficient time for bean beetle feeding on *P.*

lunatus lines. The mean of 62 and 80 days, or 71 days, would be more nearly right for the final evaluation when attempting to compare *Phaseolus* spp. and related genera relative to bean beetle feeding injury.

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The Effects of Some Insecticides on the Metabolites of *Blattella germanica*[1]

HUBERT B. CLARK and ANDREW BUTZ,

Department of Biological Sciences, University of Cincinnati, Cincinnati 21, Ohio

ABSTRACT

A study was made of the effects of some insecticides on the metabolites of adult German cockroaches (*Blattella germanica* (L.)), by comparing the glucose, glycogen, fat, and water constituents of normal and exposed whole insects.

Pieces of filter paper were impregnated with 5% concentrations of DDT, dieldrin, methyl parathion, and Strobane® (terpene polychorinates (65% chlorine)) in peanut oil. Insects were exposed to the various insecticides by being caused to walk on the pieces of filter paper for 2-minute periods.

Insects exposed to DDT showed that 62% of glucose, 73% of glycogen, and 16% of fat were utilized in 3 days. Insects exposed to methyl parathion showed a 55%, 64%, 0% decrease in metabolites, respectively. Insects exposed to Strobane showed a 57%, 56%, 19% decrease in metabolites, whereas those exposed to dieldrin showed no change.

There is thus some evidence to support the hypothesis that a reduction in metabolites is associated with the killing action of some insecticides, but also, that not all insecticides have this effect. Evidently dieldrin kills by some mechanism different from that operative with the other insecticides tested.

The purpose of this investigation is to determine the changes that occur in the metabolite composition of the German cockroach (*Blattella germanica* (L.)) as a result of exposure to DDT, dieldrin, methyl parathion, and Strobane® (terpene polychorinates (65% chlorine)), and to compare the results with that of other investigators on starvation, where energy-furnishing metabolites are lost, and insecticidal contact in other insects.

MATERIAL AND METHODS.—Adult *Blattella germanica* were obtained from the departmental animal room by catching them in funnel cockroach traps containing karo syrup. The cockroaches were taken from the traps and placed in culture dishes where they were given an abundant supply of food and water. For each analyzed metabolite, 10 separate tests were made and an average was taken. Chemical analyses were made on normal German cockroaches and on those which had been exposed to 5% concentration of insecticides. The insecticides (obtained from Nutritional Biochemicals Corporation) were dissolved in refined peanut oil in such a manner as to give a 5% concentration of each insecticide. Dissolved samples of insecticides were placed on filter papers and rotated in a glass dish until the paper became thoroughly impregnated. Test insects were taken from the culture dishes and confined for 2 minutes on the treated filter paper which completely covered the test area. An inverted petri dish was used to keep the insects confined within the test area. After 2 minutes of exposure the insects were placed in separate labeled culture dishes containing ample supplies of food and water. Preliminary experiments indicated that the insects lived approximately 5 days after such exposure to each insecticide, therefore chemical analyses were made 3 days following exposure in order that they would be alive at the time of analysis.

Determination of reducing substances, expressed as glucose, were made on the entire insects, by Folin-Malmros (1929) analysis. Glycogen was precipitated by a modified Pflüger technique (Good et al. 1933). Determination of fat was made by ether extraction in a Soxhlet apparatus; this procedure gave values for free, or ether extractible, fat. Water content was determined by weighing before and after complete vacuum desiccation, and determining the difference.

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Table 1.—Metabolites and water content of normal cockroaches.

Substance	Weight of Substance	Body Weight	
	Average Weight (mg.)	Average Weight (mg.)	Per Cent of Body Weight
Glucose	0.37	61.35	0.60
Glycogen	0.62	84.90	0.70
Fat	7.53	79.90	9.00
Water	55.40	79.90	69.00

OBSERVATIONS.—The metabolites and water content of normal German cockroaches are given in table 1. Since preliminary analyses had shown that there was no great difference between male and female cockroaches as far as glycogen, glucose, fat, and water content are concerned, the data are presented without reference to sex.

Table 2 gives the average analysis of metabolites and water content of cockroaches exposed to 5% solutions of DDT, dieldrin, methyl parathion, and Strobane, respectively. Cockroaches exposed to 5% DDT solution for 2 minutes showed a decrease in glucose from 0.60% to 0.23% of body weight. The relative amounts of glycogen and fat also decreased from 0.70% to 0.19% and 9.00% to 7.50%, respectively. The percentage of water decreased from 69% to 58% of body weight. Dieldrin-exposed cockroaches showed no decrease in metabolites but water loss occurred. Cockroaches exposed to methyl parathion solution showed a decrease in glucose and glycogen content closely paralleling the results obtained for DDT-exposed insects, but showed no apparent fat loss. The percentage of water loss is in range with the results observed for DDT and dieldrin. Strobane-exposed cockroaches showed a decrease in all the metabolites. The loss of fat was smaller but observable. The percentage of water loss is consistent with those previously described.

DISCUSSION.—In DDT-exposed *Phormia regina*, Buck & Keister (1949) found more respirable substrates were left than in starved controls. However, a comparison of Ludwig (1946) with that of Newton (1954) on the larva of *Popillia japonica* indicated that more of the available carbohydrates were used during exposure to DDT than during starvation. Newton showed a loss of glucose to be 28.6%, glycogen 90.0%, and fat 71.7% in the starved insect and Ludwig indicated a loss of 40.4%, 85.5%, and 32.8%, respectively, in DDT-exposed insects. From these results, carbohydrate loss is greater during the period following DDT exposure than starvation. In a later work by Ludwig & Bartolotta (1953), it was suggested that DDT might interfere with an enzyme system needed for fat utilization and so there is a greater use of carbohydrates as an energy source.

Table 2.—Percentage change of metabolites and water content of exposed cockroaches.

Substance	Per Cent of Body Weight				
	Normal	DDT	Dieldrin	Methyl Parathion	Strobane
Glucose	0.60	0.23	0.62	0.27	0.26
Glycogen	0.70	0.19	0.88	0.25	0.31
Fat	9.00	7.50	11.00	9.70	8.30
Water	69.00	58.00	47.00	61.00	48.00

The present investigation on the effects of DDT on the metabolites of adult German cockroaches yields evidence that DDT has an adverse effect upon their metabolism. In the present experiment 62% of the original glucose was utilized in 3 days following DDT exposure; 73% of glycogen and 16% of fat were also utilized. These figures are in close relationship with the findings of Ludwig (1946). The loss may be accounted for by a disturbance of the integument due to the corrosive action of DDT or extreme respiratory exchange. It appears that fuel metabolite loss which follows DDT poisoning is the cause of death. The works of Beard (1957, 1958) on the effect of DDT on the larvae of *Galleria mellonella* (L.) confirms this conclusion. Beard stated that during DDT toxication, the insect depleted a great quantity of reserve metabolites during hyperactivity and probably died of physical exhaustion. Prompt and complete recovery from DDT could be obtained when energy-rich substances were administered to the insect or death was delayed when loss of ATP was prevented by blocking muscle action with braconid venom.

The modes of action of methyl parathion and Strobane were similar to that of DDT. There is no doubt that a major biochemical lesion in insects exposed to these compounds is the disturbance in metabolism. Harvey & Brown (1951) observed that injected parathion caused a marked increase in the respiration of adult German cockroaches during the hyperactive stage, and that the respiration was reduced when the insects became paralyzed. In the present experiment, 55% of the original glucose was utilized, 64% of the glycogen, and none of the fat, following methyl parathion exposure. A water loss comparable to that of DDT-poisoned insects was observed. Strobane-exposed cockroaches showed a reduction of glucose, 57%; glycogen, 56%; and fat, 19%. With the common modes of action of DDT, Strobane, and methyl parathion, it may be concluded that these compounds have similar effects on carbohydrate metabolites. DDT and Strobane have similar effects on fat metabolism while methyl parathion does not. This may be due to the fact that the more rapid paralysis of the insect which is brought about by methyl parathion toxication prevents the utilization of the fat.

Dieldrin had no measurable effect upon the metabolites, but there was nevertheless a water loss similar to those of insects exposed to the other insecticides. It may be postulated that the killing action of dieldrin is by a mechanism different from that of the other insecticides used.

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Mites on Soybeans in Delaware[1]

J. E. BAKER and W. A. CONNELL[2]
Delaware Agricultural Experiment Station, Newark

ABSTRACT

In a systematic statewide survey of soybean fields during the years 1958–60, three species of phytophagous mites were collected, but only one, *Tetranychus atlanticus* McGregor, the strawberry spider mite, was found frequently enough to be considered an economic pest. The others were *Tetranychus lobosus* Boudreaux and *Panonychus* sp. In 1960, 26% of all leaves collected were infested with spider mites. *T. lobosus* was present on only 2% of the leaves and *Panonychus* sp. on only one leaf. Other mites encountered were a predaceous *Typhlodromus* sp., and a possibly predaceous *Balaustium* sp., as well as *Pronematus* sp., *Tyrophagus* sp., *Tarsonemus* sp., and *Sejus* sp., all of which may be saprophagous. Efforts to relate fluctuations in *T. atlanticus* populations to rainfall gave inconclusive results.

There has been a phenomenal increase in soybean production in Delaware during the past 20 years. In 1940 this was a minor crop, grown here on about 45,000 acres. By contrast, approximately 184,000 acres were planted to soybeans in 1960. This acreage exceeded that planted to corn and was about 35% of the land under cultivation in the State.

With this increase in soybean acreage, a suitable environment for soybean pests has also been greatly enlarged. Among such pests are spider mites, the populations of which have developed to injurious proportions in certain years. Milliron (1958) states that in 1953, 1954 and 1955 severe infestations of these mites resulted in as much as 50% foliage drop in some fields. Our Economic Insect Pest Survey records (unpublished) show a similar situation in 1957.

In 1958 a systematic statewide survey was initiated, the objectives of which were to determine the species of mites occurring on soybeans, their relative importance as pests of this crop, the annual and seasonal fluctuations in their abundance and the possible relation of rainfall, parasites and predators to these fluctuations. Each year 10 fields, at as many different locations, were visited weekly and collections of six leaves from as many plants per field were made at each visit. The leaves were taken at random from various positions on plants selected at random from among those growing on or near the field margin. They were placed in small plastic bags and brought back to the laboratory for examination. This study consisted of counting, with the aid of a stereoscopic microscope, the active mites and eggs that were found.

Table 1.—Percentage of soybean plants infested with phytophagous mites along margins of 10 fields, in Delaware, July through September.[a]

LOCATION	YEAR		
	1958	1959	1960
Frederica	8.3	14.9	16.6
Milton	0.0	33.3	44.9
Harbeson	0.0	20.0	16.6
Millsboro	49.8	24.9	13.2
Knowles	25.6	16.6	33.2
Laurel	49.8	34.9	31.5
Bridgeville	4.9	39.9	3.3
Farmington	28.1	36.5	21.5
Felton	8.6	45.0	33.2
Cheswold	6.6	31.5	48.1
Average	18.2	29.8	26.2

[a] Based on one leaf per plant and sixty plants per field.

Leaves were collected from about the first week of July until about the end of September, so that in each year there were 10 collections from each field, or a total of 600 leaves. At most locations the same fields were visited in each of the three years. In those few cases where a field, examined in one year, did not have soybeans the following year it was possible to find another soybean field within less than one-fourth mile.

In the 3 years that the study has been in progress, the spider mite infestation of soybeans has been relatively light. Only occasional fields have shown conspicuous signs of injury. Although all of the leaves collected from some of the fields were infested on certain occasions, these high populations soon disappeared, as will be discussed below. In table 1 the average infestation for each field in each year is listed. In those cases where these data show 40% or more of the leaves infested, mite injury to the foliage was noticeable. It is noteworthy that no more than two of the 10 fields fall in this category each year.

The species of mites collected are listed in table 2. The strawberry spider mite, *Tetranychus atlanticus* McGregor,

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[2] Graduate assistant and associate professor, respectively. W. N. Cannon, now at Ohio Agricultural Experiment Station, assisted with the 1958–59 survey.

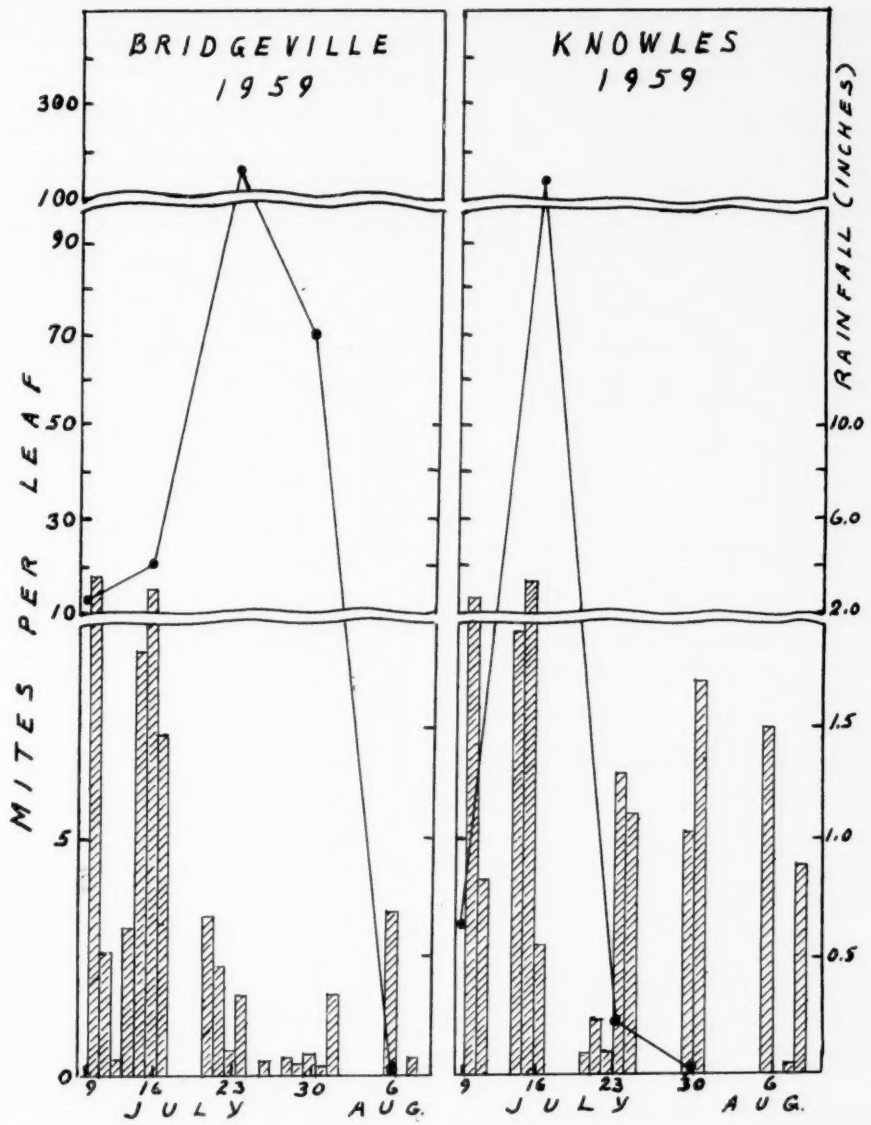

FIG. 1.—A comparison of population changes of *Tetranychus atlanticus* McG. (line graph) with precipitation (bar graph) at two locations in Delaware, 1959.

was the most common one. Although Milliron (1958) reported both this and *Tetranychus telarius* (L.) as occurring frequently on soybeans, we did not encounter the latter species. There was a single collection containing a male which may have been *telarius*, but the determination was doubtful.

These two species can be separated with certainty only through examination of the male genitalia. Many times infested leaves were encountered that had no males on them. During the first 2 years these were simply recorded as infested. However, *T. atlanticus* was identified from 36.8% and 30.2% of the infested leaves in 1958 and 1959, respectively. In 1960, when males were not present at the time of the initial examination, rearing of these was attempted by placing the infested leaves on wet filter paper in petri dishes. As a result of adoption of this technique, it was possible to determine the species present on 82.1% of the infested leaves collected. *T. atlanticus* was found on 74.5% while 7.6% were infested with *Tetranychus lobosus* Boudreaux, a species previously unreported from the State.

Of the other species identified and listed in table 2,

Table 2.—List of mites collected on soybean plants in Delaware, 1958–1960.

SPECIES[a]	FEEDING HABIT
Tetranychus atlanticus McG.	phytophagous
Tetranychus lobosus Boudr.	phytophagous
Panonychus sp.	phytophagous
Typhlodromus sp.	predaceous
Balaustium sp.	possibly predaceous
Pronematus sp.	possibly saprophagous
Tyrophagus sp.	possibly saprophagous
Tarsonemus sp.	possibly saprophagous
Sejus sp.	habits unknown

[a] Determinations by, or confirmed by, E. W. Baker, U. S. Department of Agriculture.

data were not recorded on abundance or frequency of occurrence. However, the predatory *Typhlodromus* sp. was encountered rather frequently, while the others were seen only occasionally. All were from leaves on which either *T. atlanticus* or *T. lobosus* were present. The *Panonychus* sp. was taken on only one leaf and this was during the last week of September, 1960.

In most of the fields where *T. atlanticus* was found, there was a gradual increase in its abundance. This was followed by a more rapid increase to a peak, after which there was a rather abrupt decline. In some cases a second increase in population occurred, while in others, mites could no longer be found in the field. In several instances where rather high populations were reached at the peak, many dead individuals could be seen on the leaves the following week. The cause of this mortality is still under investigation.

There is a widespread belief among soybean growers that hot dry weather favors mite infestations on this crop, and that rainy periods wipe them out. It is possible that atmospheric moisture does influence the population level of the strawberry spider mite, but we could not find a consistent correlation with precipitation. Populations of *T. atlanticus* were compared graphically with rainfall data supplied by the United States Weather Bureau Station nearest to each of the fields in this study. The two examples of these charts, given in figure 1, indicate the trends that were noted. In some cases, Knowles 1959 for example, population decline took place during or immediately after a period of heavy rainfall. In other instances, for example Bridgeville 1959, the population continued to rise for 1, 2 and even more weeks after the onset of heavy rains. At Knowles the rain gauge was on the same farm as the soybean field under consideration; at Bridgeville the rain gauge was about 2 miles from the field.

Further study of the relation of moisture to populations of *T. atlanticus* is being planned.

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Introduction of European Parasites of *Sitona* spp. for Control of the Sweetclover Weevil, *Sitona cylindricollis*, in Canada.[1]

CONRAD LOAN[2], *Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario*

ABSTRACT

European insect parasites of adult *Sitona* spp. were released in Manitoba, Canada, against the sweetclover weevil, *Sitona cylindricollis* Fåhraeus. The braconid *Pygostolus falcatus* (Nees) was reared from *S. cylindricollis* for 2 successive years following its release at Brandon. There is no evidence that either of the braconids *Microctonus aethiops* (Nees) auct. or *Perilitus rutilus* (Nees) or the tachinid *Campogaster exigua* (Meigen) are established.

During studies at Belleville, Ontario, on the development of *S. cylindricollis* summer-emerged adults were observed to mate and oviposit without a period of diapause at a low temperature. The weevils developed more successfully on sweetclover than on other species of legumes and development was significantly more rapid on *Melilotus officinalis* (L.) Lam. than on *M. dentata* Pers.

The sweetclover weevil, *Sitona cylindricollis* Fåhraeus, (fig. 1) is a major economic pest of sweetclover, *Melilotus* spp., in Canada (Bird 1947, 1950) and the United States (Herron 1953), and of alfalfa, *Medicago sativa* L., in the Soviet Union (Yakhontov 1935). The adult weevils attack the foliage (fig. 2A) and in the spring may destroy sweetclover in the cotyledon stage. The larvae attack the root system (fig. 2C, D) and nodules of sweetclover. Bird (1950) found that weather and the fungus *Beauveria bassiana* (Bals.) Vuill. were the chief factors regulating the abundance of *S. cylindricollis* in Manitoba, but he found no insect parasites of the adult weevil and none is known to occur in Europe or Asia. The braconid, *Microctonus sitonae* Mas., is a parasite of *S. scissifrons* Say in Canada but not of *S. cylindricollis* (Loan 1960, and unpublished data). Parasites of other *Sitona* species were recorded in Europe by Jackson (1920, 1922, 1924, 1928), Meyer (1934), Ulashkevich (1935), Grossheim (1928), and Berry & Parker (1950). Initial attempts by Munro & Post

[1] Accepted for publication April 25, 1961. This paper was endorsed and communicated by Dr. Bryan P. Beirne, Director, Entomology Research Institute for Biological Control, Canada Department of Agriculture.

[2] The early phase of this work was undertaken by Mr. R. W. Smith, Entomology Research Institute for Biological Control, Belleville, Ontario. The writer is grateful to Dr. J. S. Kelleher of this Institute (formerly of Canada Agriculture Research Station, Winnipeg) for releasing the parasites and collecting sweetclover weevils in Manitoba and to the following individuals for parasites and weevils: Dr. H. L. Parker, European Parasite Laboratory, U.S.D.A., Paris, France; Mr. S. Johansson, Zoological Institute, University of Lund, Lund, Sweden; Dr. V. V. Yakhontov, Academy of Sciences, Tashkent, Uzbekistan, U.S.S.R.; Dr. K. Morimoto, Kyushu University, Fukuoka, Japan; and Mr. Olaf Wagn, Statens Plantepatologiske Forsøg, Lyngby, Denmark. Appreciation is extended to Mr. W. J. Brown, Dr. W. R. M. Mason, and Mr. R. de Ruette, Entomology Research Institute, Ottawa, for identification of specimens; to Dr. F. G. Holdaway, University of Minnesota, St. Paul, for advice on many aspects of the work; to Miss Dorothy Jackson, North Cliff, St. Andrews, Scotland, for information on parsites and weevils; to Dr. K. Smith, University of Wisconsin, Madison, for seed of *Melilotus dentata;* and to Mr. Harold Smith, R.R. 2, Brandon, Manitoba, for assistance with the parasite releases.

(1948) to colonize European parasites in North Dakota were followed in Canada by a program to obtain, study, and release parasites of *Sitona* spp. This paper reports the methods and results of attempts to colonize three braconids, *Microctonus aethiops* (Nees) auct., *Perilitus rutilus* (Nees) and *Pygostolus falcatus* (Nees), and a tachinid, *Campogaster exigua* (Meigen), with observations on the biology of the sweetclover weevil and the parasites.

BIOLOGY OF *S. cylindricollis*.—The life history of *S. cylindricollis* in Manitoba was discussed by Bird (1947, 1950), in Ohio by Herron (1953), and in Central Asia by Yakhontov (1935). In Manitoba the weevils emerge from hibernation in the spring and disperse by running and flying. They mate and oviposit from spring emergence to mid-summer, but high surface temperatures and desiccation destroy a majority of larvae that hatch in July. The eggs (fig. 3) are laid at random and they drop to the surface of the soil. Four larval instars develop in the soil on nodules and root hairs. The fourth-instar larva pupates near the soil surface. Weevils of the new generation emerge in late July and in August and overwinter as sexually immature adults in the surface layer of soil. The weevil has one generation in Manitoba and elsewhere in North America, but has two generations in Central Asia (Yakhontov 1935).

Herron (1953) found that weevils taken from hibernation failed to oviposit before March, and Davey (1956) referring to maturation of the ovaries stated that "a period of exposure of a low temperature of more than 100 days is necessary to terminate the dormancy in *Sitona*." Weevils reared in an insectary at Belleville in the summer of 1955 during studies of the European braconid parasites mated and oviposited at 72° F. in the greenhouse in January, 1956, without an intervening period of low temperature. The weevils were taken from the insectary on July 21, 1955, to the laboratory at a constant temperature of 74° F. with normal day-lengths, and from there to the greenhouse on December 13. The temperature in the greenhouse fluctuated widely over the 72° F. minimum because of the heat of the sun. Ten females laid from 10 to 568 eggs each (average 304.0) from January 25 to March 20, and one female failed to oviposit. Other weevils, of Manitoba origin, taken from field cages near Belleville to the greenhouse on February 9, 1956, mated 4 days later and oviposited on February 15. In 1959, summer-emerged, sexually immature weevils collected in the field in September mated and oviposited at a constant temperature of 74° F. in early October.[3]

During February and March, 1956, several species of legumes were infested with weevil eggs in the greenhouse to determine whether the sweetclover weevils would develop on legumes other than sweetclover. Approximately 300 eggs were placed at the crown of each plant and were lightly covered with moist earth. The plants were first-year growth about 6 months old. No adults were obtained from eggs placed on *Trifolium repens* L., *T. pratense* L., or *T. hybridum* L.; one adult emerged from alfalfa plants, 44 from *Melilotus dentata* Pers., and 77 from *M. officinalis* (L.) Lam. Development of the weevil from egg to adult was 82.3 ±9.85 days on *M. dentata* and 72.7 ±9.46 days on *M. officinalis*, a difference that was significant at the 1% level. Failure of the weevil to develop on *Trifolium* spp. is further evidence that *S. cylindricollis* is largely dependent on *Melilotus* spp. for increase and survival. In April, 1955, *S. cylindricollis* collected from the crowns of alfalfa at Marmora, Ontario, were fed alfalfa and sweetclover in the laboratory. Egg counts were made up to May 26, and the weevils on sweetclover produced 940 eggs and those on alfalfa, only six eggs. Yakhontov (1935) found that *S. cylindricollis* was an abundant species on alfalfa in Central Asia. Indication that alfalfa is not a preferred host was obtained when a number of living *S. cylindricollis* from Tashkent, Uzbekistan, were offered a choice of sweetclover and alfalfa in cages at Belleville. Feeding was almost entirely restricted to sweetclover. From this it seems probable that sweetclover, if grown as a forage crop in Central Asia, would be infested by *S. cylindricollis* in preference to alfalfa. The evidence suggests that the *S. cylindricollis* in Central Asia is a strain that is adapted to alfalfa, whereas in North America *S. cylindricollis* breeds and feeds on its preferred host plant, sweetclover.

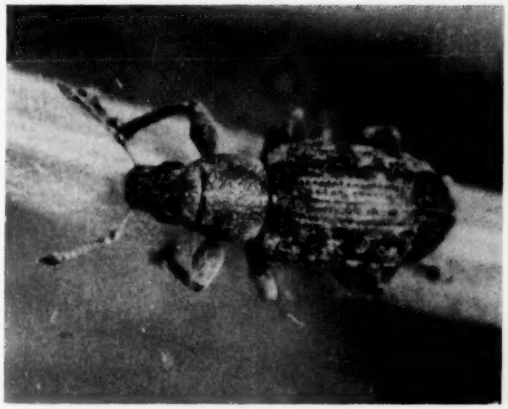

FIG. 1. *Sitona cylindricollis* Fähr. ×15

THE PARASITES.—The life-histories of the parasites were described earlier: *P. rutilus* by Jackson (1928); *P. falcatus* by Loan & Holdaway (1961a); *M. aethiops* and *P. rutilus* by Loan & Holdaway (1961b); and *C. exigua* by Berry & Parker (1950). The major host species of *P. falcatus* and *P. rutilus* in Sweden was *S. lineata* (L.) and those of *M. aethiops* and *P. rutilus* in France were *S. humeralis* Steph. and *Hypera postica* (Gyll.). The adult weevil is attacked by the parasites and superparasitism and multiparasitism occur. A preference for one or more species of *Sitona* was not evident in the laboratory. The immature parasite stages are found in the haemocoel and one larva only develops in each host weevil. Associated with the larva are the dissociate cells of the trophamnion of the embryo (teratocytes) which increase in volume in relation to the growth of the larva. The fifth-instar larva emerges from the weevil. There is no indication of parasitism 24 hours before the weevil dies. The cocoon is spun in soil (*M. aethiops*, *P. rutilus*) or on foliage and other

[3] Dr. F. G. Holdaway has kindly forwarded to me a copy of a manuscript in press (Ent. Exptl. & Appl.) by Dr. Heidewig Hans entitled "Termination of Diapause and Continuous Laboratory Rearing of the Sweetclover Weevil *Sitona cylindricollis* Fahr." in which it is demonstrated that diapause is terminated by a definite photoperiod or low temperature, or may be circumvented in summer-emerged weevils.

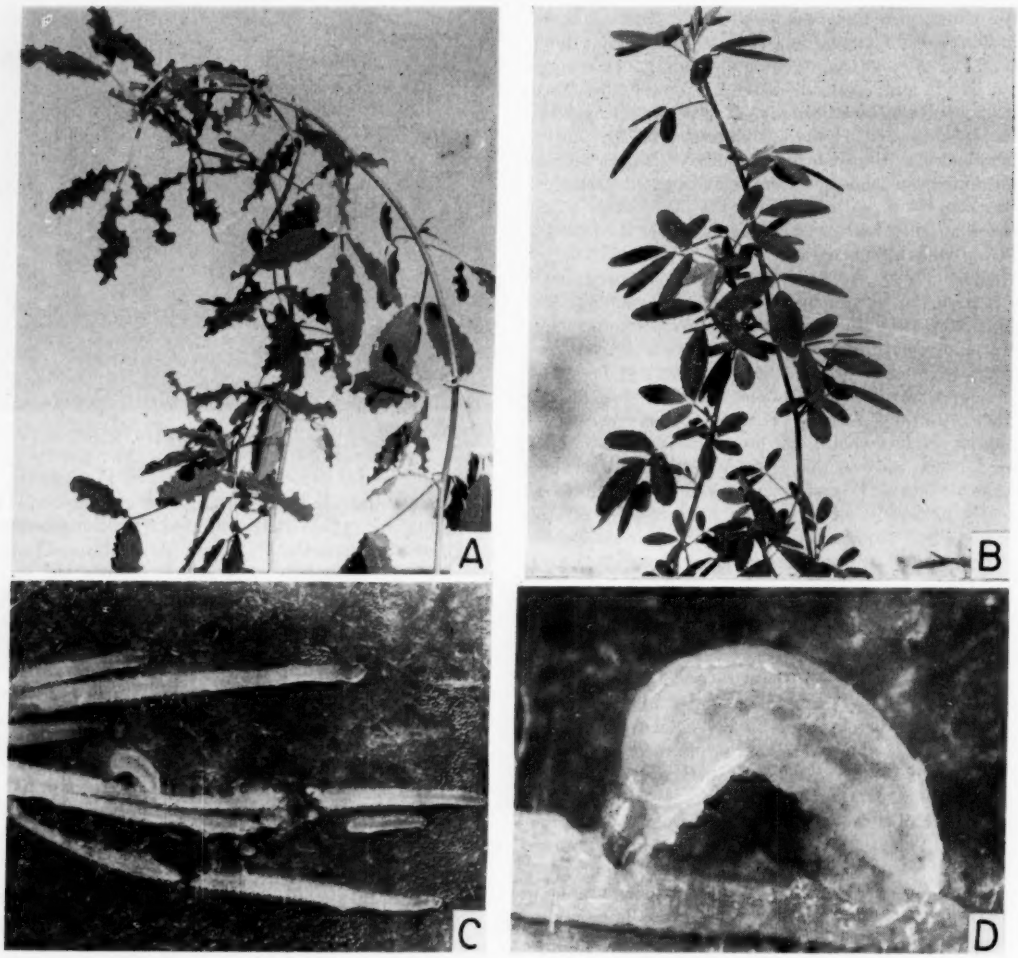

FIG. 2.—Injury by *S. cylindricollis:* A, injured foliage of sweetclover; B, normal foliage; C, roots of sweetclover bisected and damaged. ×1.5; D, larva feeding on root. ×20

places in the weevil habitat (*P. falcatus*). The immature parasite stages, the cocoons and a parasitized weevil are shown in figure 4. The braconids overwinter as first-instar larvae in summer-emerged weevils. Parasites that develop from the overwintered parasite larvae attack the surviving overwintered weevils. In Sweden parasitism of *S. lineata* (L.) by *P. falcatus* and *P. rutilus* is greatest from mid-June to July. In France parasitism of *S. humeralis* and *H. postica* by *M. aethiops* is greatest from April to May. A second generation of parasites attacks the summer-emerged weevils. For the most part the parasites remain in summer-emerged weevils as first-instar larvae in diapause and resume their development after they have overwintered.

Microctonus aethiops and Campogaster exigua from France.—*M. aethiops* and *C. exigua* were sent air express from Paris to Montreal via New York, thence by rail to Belleville—a period of travel of about 3 days.

M. aethiops was shipped as larvae or pupae in cocoons, in glass or plastic vials packed in excelsior in a small wooden box. The cocoons of *M. aethiops* were incubated in quarantine at 74° F. As the adults emerged they mated and fed upon honey and water.

C. exigua was collected in the field as adults and these were shipped in a wooden container with strips of cloth for resting-sites and drops of agar-agar-honey solution on paper for food.

Both species were shipped to the release point at Portage la Prairie, Manitoba, by rail express in a refrigerated box. Very few *M. aethiops* died en route but the mortality of *C. exigua* was high both en route from France and en route to the release point. Details of the release are given in table 1.

Pygostolus falcatus and Perilitus rutilus from Sweden.—The parasites of Swedish origin, *P. falcatus* and *P. rutilus*, were obtained from 1956 to 1959. They were reared from living adults of *S. lineata*, *S. humeralis* and *S. hispidula* (F.) in June and July. The Swedish collections

Table 1.—Number and species of parasites released at Portage la Prairie, Manitoba, 1952–1954.

Date of Release	Number of			
	Microctonus aethiops		*Campogaster exigua*	
	Males	Females	Males	Females
May 22, 1952	335	425	11	54
May 8, 1953	264	351		
May 25, 1953	156	236		
August 1, 1953			1	10
May 31, 1954	134	193		
Totals	889	1,205	12	64

were made near Kattarp, southern Sweden, and the weevils were shipped by air express from Copenhagen via London to Montreal and thence by rail to Belleville—a period of travel of about 4 days. The weevils were forwarded in shipping containers of the type described by Delucchi (1958), with loose foliage of vetch or alfalfa, and arrived with little or no mortality. They were placed in quarantine upon arrival, identified, and then held in cages at 74° F. for emergence of the parasite larvae. Larvae of *P. rutilus* crawled through the mesh floor of the cage to form their cocoons in soil, or on black cloth. The larvae of *P. falcatus* formed their cocoons on the walls of the cage or on foliage. As the adults emerged they were placed in small, wooden cages and provided with pure honey and water. Without honey *P. falcatus* died within 1 or 2 days.

P. falcatus and *P. rutilus* were released in Manitoba, in 1958 and 1959, as first-instar larvae in summer-emerged weevils. The weevils were swept near Brandon, Manitoba, and forwarded to Belleville by air express for parasitism. These were exposed in lots of 100 to 400 to 25 to 50 parasites for 12 to 24 hours. Of a total of 18,200 weevils exposed to *P. falcatus*, sample dissections indicated that 5,000 to 7,000 weevils were parasitized. Of a total of 5,000 weevils exposed to *P. rutilus* not more than 750 to 1,000 weevils were parasitized. The exposed weevils were returned to Manitoba by air express and released as shown in table 2.

Release Points.—The main requirements of the parasite release points were stands of first-year sweetclover as a source of food and as overwintering sites for the released

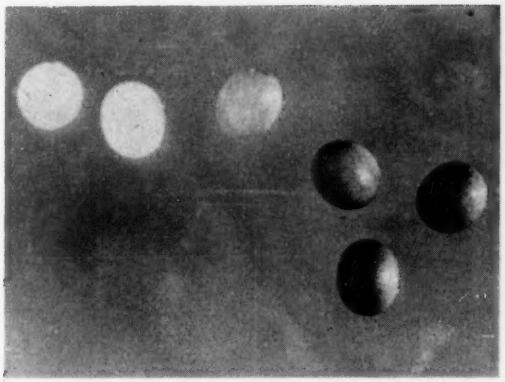

FIG. 3.—Eggs of *S. cylindricollis*. The white eggs are newly laid and the dark eggs are 24 or more hours old. ×24

weevils, and a large population of *S. cylindricollis*. A desirable feature was the occurrence of alternate host species, but none of these occurs on sweetclover and only one other weevil, *S. scissifrons* Say, occurs in the region, and this on vetch and alfalfa. Suitable locations were difficult to find in 1958 because of poor stands of sweetclover. Early spring weather favored sweetclover development, but lack of rainfall delayed seeding and growth was greatly retarded by dry weather in June and July (J. S. Kelleher *in litt.*). As a result of this poor growth, the field of sweetclover at Oakville, Manitoba, where the parasitized weevils were released, was tilled in the fall of 1958 and collections of weevils for evidence of parasite establishment were made the following year 3 miles southwest of the liberation field. The field of sweetclover at Portage la Prairie, Manitoba, where parasites were released in 1958 was also tilled in the fall except for some 20 acres of sweetclover 500 to 600 yards north of the release point. The stand of sweetclover at Brandon where the parasitized weevils were released in 1958 was not disturbed by tilling, though growth was thin in places.

Assessing Establishment.—Parasite establishment was assessed by dissection and rearing of weevils. In 1952 and 1953, small numbers from the release points were dissected. In 1954, 6,000 overwintered and 15,000 summer-emerged weevils were collected at Portage la Prairie and

Table 2.—Number and species of parasites released as first-instar larvae in summer-emerged adults of *S. cylindricollis* in Manitoba, 1958–1959.

Parasite	Date of Release	No. Weevils Released	Per Cent Parasitism	Release Point in Manitoba
		1958		
Pygostolus falcatus	Aug. 6	8,000	31–43	Brandon (Harold Smith farm)
Perilitus rutilus	Aug. 6	2,000	15–20	
Pygostolus falcatus	Aug. 8	4,600	22–32	Portage la Prairie
Perilitus rutilus	Aug. 8	1,000	15–20	
Pygostolus falcatus	Aug. 22	5,600	26–35	Oakville
Perilitus rutilus	Aug. 22	2,000	10–12	
		1959		
Pygostolus falcatus	Aug. 26	1,000	25	DuFrost

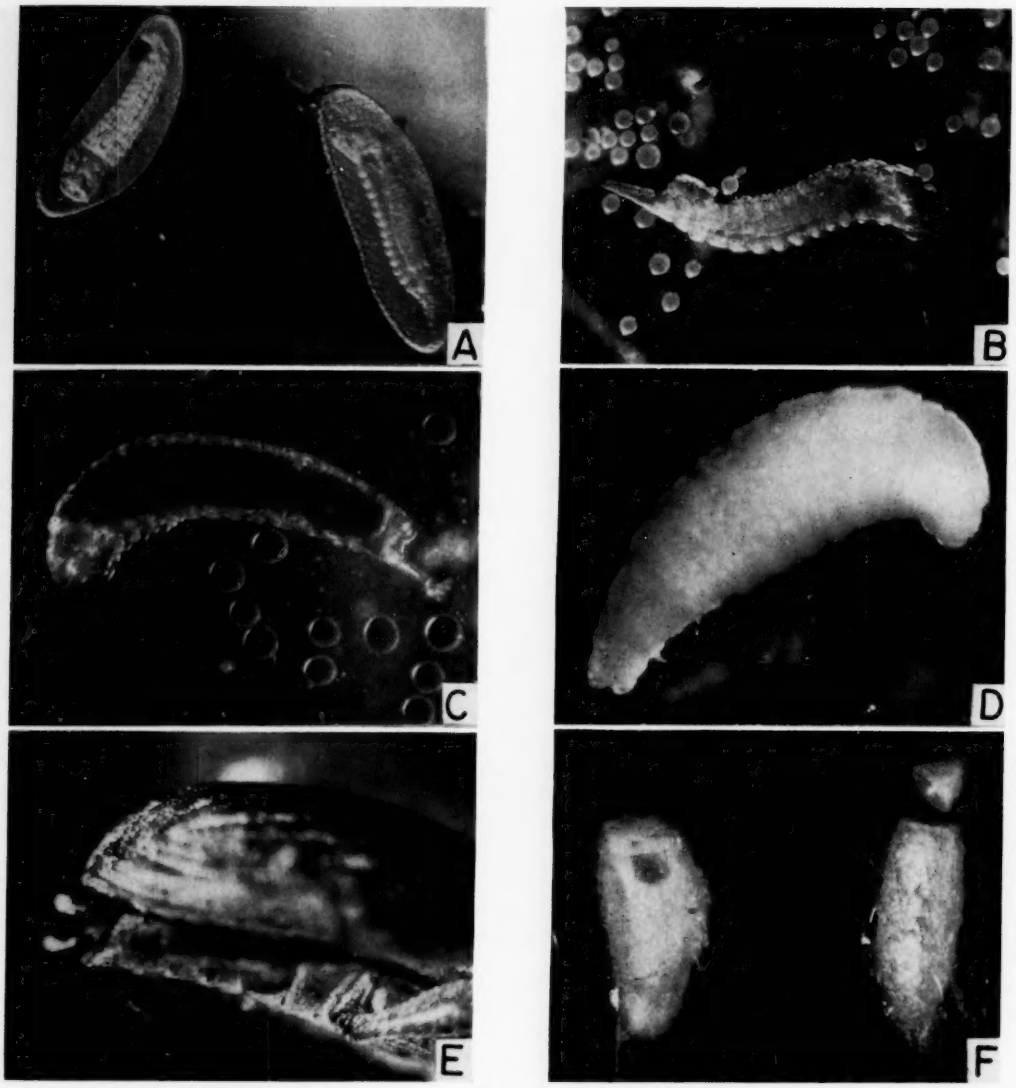

FIG. 4. Life history of the parasites. A–D, immature stages of *Pygostolus falcatus:* A, mature egg. ×43; B, instar I larva and teratocytes. ×25; C, instar III larva and teratocytes. ×25; D, instar IV larva. ×25; E, apex of abdomen of *S. cylindricollis* showing effect of emergence of *Microctonus aethiops;* F, cocoons: left, *Perilitus rutilus* (emergence opening near top center), right, *Pygostolis falcatus.* ×9.5

held for parasite emergence. In 1959, 90,000 overwintered weevils and 75,000 summer-emerged ones were swept from the 1958 release points and reared or dissected. In 1960, 3,120 overwintered *S. cylindricollis* were swept on June 13–14 from sweetclover at or near the 1958 and 1959 release points. The collections were examined for adult parasites.

RECOVERIES.—No larvae of *M. aethiops*, *P. rutilus*, nor *C. exigua* were reared or dissected from *S. cylindricollis*. *P. falcatus* was recovered in 1959 and 1960 from the Brandon release but not from those at Portage la Prairie, Oakville, or DuFrost. From about 4,500 hosts collected at Brandon on June 15, 1959, 24 larvae of *P. falcatus* emerged from June 27 to July 3. Of 520 weevils dissected, six were parasitized: two with mature eggs, one with a first-instar larva, and three with advanced instar larvae. The second Brandon collection on June 26, of 7,000 weevils, yielded no parasites, but six larvae emerged from 10,000 hosts collected 1 week later. No additional parasites were reared or dissected from large numbers of overwintered weevils collected at the release point and at distances of ½, 2, and almost 10 miles away from it. Parasitism of summer-emerged weevils was not determined because the weevils died in cold storage.

In 1960, three larvae of *P. falcatus* emerged from 2,302 weevils swept about 600 yards northeast of the 1958 Brandon release point. A larva of a *Microctonus* species, probably *sitonae* emerged from 389 *S. scissifrons* collected south of St. Pierre de Jolys, Manitoba, from *Vicia* and *Astragalus* species.

DISCUSSION.—*P. falcatus* is not known to attack *S. cylindricollis* in Europe, yet it has parasitized *S. cylindricollis* and overwintered at Brandon for 2 successive years since its release in the fall of 1958. It emerged in the spring of 1959 and parasitized overwintered weevils of the same generation in June. Although the incidence of parasitism of summer-emerged weevils was not known in 1959, a proportion of them yielded *P. falcatus* in the spring of 1960. Thus the low weevil population in July that normally occurs between generations was not a barrier to the establishment of *P. falcatus*.

Biological differences between the braconid species favor *P. falcatus*, which is parthenogenetic and thelyotokous whereas *M. aethiops* and *P. rutilus* are arrhenotokous. This is obviously favorable to *P. falcatus*, as a single female may start a colony and carry the species from one generation to the next. If, for any reason, females of *M. aethiops* or *P. rutilus* are not fertilized, a colony of the parasites would not survive. Adult *M. aethiops* and *C. exigua*, released in May of 1952–1954, were expected to go through one or two generations in overwintered weevils and parasitize summer-emerged weevils in late summer for overwintering. The apparent failure of this sequence of events may be attributed partly to some reaction in *S. cylindricollis* that results in the death of a high proportion of the first-instar larvae of *M. aethiops* (Loan & Holdaway 1961b). On the other hand, *P. rutilus*, too, failed to survive in 1958 though its development in the sweetclover weevil was normal. The Oakville and Portage la Prairie release points were tilled in the fall of 1958 and this might have killed some of the released weevils and forced others to disperse. The DuFrost site was unfavorable to the establishment of *P. falcatus* in 1960 because the population of *S. cylindricollis* was low, with an average of 0.09 weevils per sweep.

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The Angoumois Grain Moth, *Sitotroga cerealella*, and Storage Fungi[1]

C. P. Misra, C. M. Christensen and A. C. Hodson[2]

Abstract

Storage fungi, principally *Aspergillus amstelodami*, *A. repens*, and *A. ruber*, in the *A. glaucus* group, were consistently isolated from nonsurface-disinfected and surface-disinfected larvae, pupae, and adults of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), and from the alimentary tract of larvae; excreta of the insect contained up to almost a billion spores of these fungi per gram. Adult moths were introduced into almost mold-free grain conditioned to moisture contents of 10.5% to 15.2%; as the insects multiplied the moisture content of the grain increased (as much as 5% in 3 months) and the population of the fungi increased greatly. Adult moths were attracted to air passed through moldy grain in preference to air passed through clean grain or to air alone, and preferred grain invaded by members of the *A. glaucus* group to clean grain.

During the last few years Agrawal et al. (1957), van Wyk et al. (1959), and Griffiths et al. (1959) have shown that a close and constant association exists between some of the grain-infesting insects and mites, and the microflora of infested grain. It seemed worth while to determine whether similar relationships might exist between the Angoumois grain moth (*Sitotroga cerealella* (Olivier)) and microflora, especially storage fungi.

Materials and Methods.—Moths were obtained from a sample of naturally infested grain that had been stored in Cairo, Illinois. Stock cultures were maintained on wheat conditioned to a moisture content of 14% to 15% in jars covered with screen mesh caps and kept at a relative humidity of 75% and a temperature of 25° C. Moisture contents of grain samples were determined by the 2-stage air oven method specified by Cereal Laboratory Methods (1957), or by drying 10 g. of grain to constant weight of 104 C. In preliminary tests the two methods gave essentially equal results. Moisture contents are given on a wet-weight basis. Grain was conditioned to various moisture contents by adding the required amount of distilled water, storing the grain in closed jars at 5° C. for 5 to 7 days, and shaking the jars vigorously occasionally to facilitate uniform distribution of the moisture. To determine number and kinds of fungi in samples of grain, in insects, or frass a weighed amount, usually 5 g., was comminuted 1.5 minutes in 500 ml. of sterile 0.12% agar solution in a Waring Blendor. Five ml. of this suspension was placed in 45 ml. of the same suspension medium in a milk dilution bottle, shaken briskly 100 times, further diluted when necessary and 1 ml. of one or more dilutions put in each of two petri dishes. Malt agar containing 7.5% or 10% sodium chloride cooled to 50° C. was added, the dishes swirled to distribute the material uniformly, the agar allowed to harden and the dishes incubated in the laboratory until colonies could be counted and identified.

Increase in Moisture Content and Storage Fungi in Wheat Free of Insects or Infested with the Angoumois Grain Moth.—Wheat was conditioned to moisture contents of 10.5%, 14.2% and 15.2%, portions of 200 to 250 g. each were placed in pint Mason jars, the controls not inoculated with insects, each of the others inoculated with 25 adult moths, the jars closed with screen caps, and stored at 25° C. and at relative humidities approximately in equilibrium with the original moisture contents of the three different lots. Samples were removed from the one control jar and from each of three replicated infested jars at each moisture content after 1, 2, and 3 months and tested for moisture content and storage fungi. The results are summarized in table 1.

Table 1.—Moisture content of and storage fungi in 250-gram portions of wheat free of insects and inoculated with 25 grain moths and stored 3 months.

Treatment	Moisture Content After Months Shown			Colonies (thousands/g.) of Storage Fungi After Months Shown		
	1	2	3	1	2	3
	Original moisture content 10.5%—stored at 70% relative humidity					
Control	10.8	12	13.6	0–1	0–1	1
Infested	11.1	13.2	16.4	2.3	4	110
	Original moisture content 14.2%—stored at 75% relative humidity					
Control	14.4	14.9	15.0	0–1	3	8
Infested	15.2	16.3	17.8	9	103	400
	Original moisture content 15.2%—stored at 80% relative humidity					
Control	15.8	16.1	16.3	1	6	15
Infested	17.6	18.6	19.9	18	5,600	16,000

As the insects developed, the moisture content of the grain increased moderately and the storage fungi increased greatly. Practically all of the colonies of storage fungi were members of the *Aspergillus glaucus* group, principally *A. repens*, *A. amstelodami*, and *A. ruber*, although when moisture contents exceeded 16% some colonies of *A. candidus* were found, and when it exceeded 17% to 18% some colonies of *A. flavus* developed. In some samples whose moisture content exceeded 16%, *Penicillium* was the predominant fungus. There was little or no increase in *A. restrictus*, which Agrawal et al. (1957) found to be the major storage fungus associated with the granary weevil, *Sitophilus granarius* (L.). There was little relation between the original moisture content of the grain, or the relative humidity of the air in the chambers where the grain was stored, and the final moisture content of the grain. Evidently enough metabolic water was produced by the larvae feeding within the kernels to more than offset the loss of water through evaporation. Obviously, data on the moisture content necessary to support development of this insect in various materials

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[2] Formerly research assistant, Professor, Department of Plant Pathology, and Professor, Department of Entomology, respectively, University of Minnesota, St. Paul. The work here reported comprised a portion of a thesis submitted by the senior author to the graduate school, University of Minnesota, in partial fulfillment of the requirements for a Ph.D. degree.

are of little value if the moisture content is determined only at the beginning of the tests.

Agrawal et al. (1957) found that even a rather rapid flow of air of 75% relative humidity through grain in which granary weevils were developing did not prevent an increase in moisture content of the grain above that in equilibrium with the relative humidity used. We made similar tests, in which wheat at about 14% moisture content was infested with moths, then air at a relative humidity of 75% was blown through it. The moisture content of the grain soon exceeded 17% and continued to rise as long as the insects continued to develop. Some of this increased moisture content was, of course, water in the bodies of the insects themselves. However, the microflora also increased at about the same rate as in the nonaerated samples, and the microfloral population was made up of about the same species as in the nonaerated samples. This is fairly convincing evidence that the increase in moisture content in the grain was real, not just apparent.

STORAGE FUNGI ISOLATED FROM INSECTS AND FROM FRASS.—A considerable number of larvae, pupae, and adults were selected at random at various times, shaken in 1% sodium hypochlorite for 1 minute, rinsed in sterile water, and cultured. Storage fungi, principally members of the *A. glaucus* group, grew from nearly 100% of these, even from the adults. The alimentary canal was dissected from a number of surface disinfected larvae and cultured on malt-salt agar. *A. glaucus* grew from all of these, first and most heavily from the proventriculus and the portion near the rectum. Weighed quantities of frass were blendorized, diluted, and cultured; these yielded from 56 million to 980 million colonies of *A. glaucus* per gram.

COMPARATIVE ATTRACTIVENESS TO GRAIN MOTHS OF CLEAN AND MOLDY WHEAT.—Adults were tested in an olfactometer and also reared in a container that allowed a choice between clean and moldy wheat. Thirty tests, each with 20 insects, were made with the olfactometer, the choices being: air passed through clean wheat vs. air alone; air passed through moldy wheat vs. air alone; and air passed through moldy wheat vs. air passed through clean wheat. Ten tests, 20 insects per test, were made with each pair.

An average of 14 to 15 insects were attracted to the air passed through clean wheat vs. 5 to 6 to the air alone; 14 to 15 attracted to the air passed through moldy wheat vs. 5 to 6 to air alone; and 14 to 15 to air passed through moldy wheat, vs. 5 to 6 to air passed through clean wheat.

The interior of a wide-mouth gallon jar was divided into two chambers of equal size by means of a vertical partition extending up to the neck of the jar. The edges and bottom of the partition were fixed and sealed to the glass wall and bottom of the jar with melted paraffin; at the top the two chambers communicated freely. On one side of the partition was put 1 kg. of hard red spring wheat free of storage fungi and with a moisture content of 12.4%; on the other side a kg. of the same wheat moderately invaded by storage fungi and with a moisture content of 14.0%. On the basis of previous tests it was assumed that the initial difference in moisture content would not be a major factor influencing the development of the insect since once larvae became active in the grain the moisture content would increase fairly rapidly. Each of the two chambers was infested with 60 adult grain moths, all of which had access to grain on both sides of the partition. Samples were removed after 20, 45, and 75 days, and 100 kernels of each sample examined for larvae. The results are given in table 2.

Table 2.—Per cent of infested wheat kernels, originally moldy and not moldy, in adjacent chambers of the same vessel, after 20, 45, and 75 days.[a]

DAYS FROM START OF TEST	PER CENT OF WHEAT KERNELS INFESTED	
	Moldy	Not Moldy
20	29	5.25
40	36	8.5
75	49	18.0

[a] Each figure is an average of 400 kernels examined.

The moisture content and mold population of the originally drier and nonmoldy wheat were not determined at the end of the test but evidently the insects preferred the moister and moldier wheat to the drier and originally nonmoldy wheat.

DISCUSSION.—In general, the relationship between the Angoumois grain moth and storage fungi is similar to that found by Agrawal et al. (1957) between the granary weevil and storage fungi. As populations of the moth developed in wheat, the moisture content of the grain increased; this increase was not so great as was found with the granary weevil, but still amounted to 5% to 7% within 3 to 4 months. Spores of a number of the common storage fungi were carried in abundance on the outside of adult moths, when the moths emerged from moldy grain, and some inoculum was carried even within the bodies of the adults, in spite of the fact that the adults do not feed, These fungi developed in the grain as the larvae developed, and spores of the fungi were consumed by the larvae along with the interior portion of the wheat grains. The alimentary canal of nearly all larvae cultured carried large numbers of viable spores of *Aspergillus* species, and the frass had up to nearly a billion spores per gram. The odor of air passed through moldy grain was more attractive to the adult moths than the odor of air alone or the odor of air passed through clean grain. When clean and moldy samples of wheat were infested with equal numbers of adult moths and stored under like conditions, more larvae developed in the moldy grain than in the clean grain. The major fungus flora associated with the Angoumois grain moth comprised members of the *A. glaucus* group, principally *A. repens*, *A. amstelodami*, and *A. ruber*. *A. restrictus* was by no means as abundant as it was found by Agrawal et al. (1957) in grain infested by the granary weevil.

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Bionomics of the Apple Aphid, *Aphis pomi*, on Young Nonbearing Apple Trees[1]

EARL R. OATMAN and E. FRED LEGNER[2]

ABSTRACT

A study of the apple aphid, *Aphis pomi* De Geer, was conducted in 1959 on young Cortland and Red Delicious apple trees under a treated (artificial) and check (seminatural) environment. The population was highest in the check and was twice as high on Cortland as on Red Delicious. The highest number of aphids on Cortland occurred in the check. On Red Delicious they were more numerous in the treated section. Alates were most numerous the first of July and were primarily responsible for the buildup of aphids following the second aphicide application in the treated section. The aphid population reached a peak on the check trees July 14, one week before maximum populations occurred on treated trees, then decreased slowly, and disappeared by late August. Parasitism was less than 0.1%. Predators included syrphid fly and aphid lion larvae, anthocorid nymphs, and coccinellid larvae and adults of which *Coccinella novemnotata* Herbst was the most abundant. Aphid abundance (intraspecific competition) was the primary biotic factor limiting the aphid population. The increase in terminal growth on treated trees was significantly greater (1% level) than that on the check.

The apple aphid, *Aphis pomi* De Geer, the most abundant insect in apple orchards in Door County, Wisconsin, causes considerable concern among growers who have experienced its destructiveness. It is usually present throughout the growing season and is more of a problem in the northern half of the State. Serious losses may occur in commercial orchards if the aphid populations are not suppressed. Injury is caused by high populations feeding early on immature apples to produce "aphid apples," and later on more mature apples to produce russeted fruit. The most extensive loss, however, occurs when high aphid populations develop on terminals, watersprouts, and grafts. The area beneath these sites is usually covered with honeydew. A sooty mold fungus develops on the honeydew, discoloring the fruit and foliage, and adversely affecting photosynthesis, quality, and yield (figs. 1 and 2).

The highest and most persistent apple aphid populations occur on young nonbearing trees where they curl, stunt, and distort the infested terminals (figs. 3 and 4). The magnitude of this type of injury is not fully known. Preliminary studies in 1958 showed a significant difference in total terminal growth on treated trees where aphids were suppressed compared with check trees with natural populations. Significant differences were found also in bud size and length of internode between buds on terminals.

An experiment was conducted in 1959 to study both the extent of injury and the factors affecting the apple aphid populations on young trees in both a treated (artificial) and a check (seminatural) environment.

METHODS AND MATERIALS.—Investigations were conducted in a 7-year-old apple orchard on the Peninsular Branch Station at Sturgeon Bay, Wisconsin. One acre contained Red Delicious and the other Cortland. The orchard was divided into two equal sections consisting of one-half acre of each variety. One section (treated) was sprayed with 40% phosphamidon, ½ lb. plus captan, 2 lb. /100 gal. on May 26 and July 2. The other section (check) was sprayed with captan at 2 lb./100 gal. on the same dates. The sprays were applied with a high pressure sprayer with air blast attachment operating at 400 p.s.i.

Population counts were made at weekly intervals from May 26 through September 1. Random samples of 25 to 50 leaves (5/5 trees at high, and 5/10 trees at low populations) were taken from vigorously growing terminals of each variety in each section. The first terminal leaf which measured at least 1 inch across was selected for the sample. Cortland leaves invariably had greater leaf surfaces than Red Delicious leaves due to growth habit. The leaves were placed in a polyethylene bag and taken into the laboratory where they were brushed through a Henderson mite-brushing machine onto a vaseline-coated counting plate. All aphids and predators were counted with a stereoscopic binocular microscope. Adult coccinellids were recovered at the height of their population by jarring them from randomly selected trees into a beating umbrella. The counts were taken early in the morning before the adults became active.

Parasitism was studied by sampling five heavily infested terminals from each variety and section at weekly intervals during the height of the aphid population. The samples were held in rearing cartons in an outdoor screened insectary.

The effect of feeding by aphids on tree growth was measured during the dormant period. Measurements were made on 10 terminals selected at random from five trees of each variety and section.

RESULTS AND DISCUSSION.—The effect of the two environments on the apple aphid for the two varieties showed that the aphid population in the untreated check was consistently higher on Cortland than on Red Delicious trees (table 1). The population in the Cortland check was twice as high as that in the treated section. By contrast, the highest number of aphids on Red Delicious occurred in the treated section. The Red Delicious trees in the treated environment became more favorable for aphid reproduction than those in the check as shown by the data for June 30 and July 21. This was apparently due to increased and prolonged terminal growth on the treated trees since the predator population was approximately the same in both sections (table 2).

The effect of the two environments on the bionomics of the apple aphid and its predators showed that the number of aphids on the untreated trees increased steadily until

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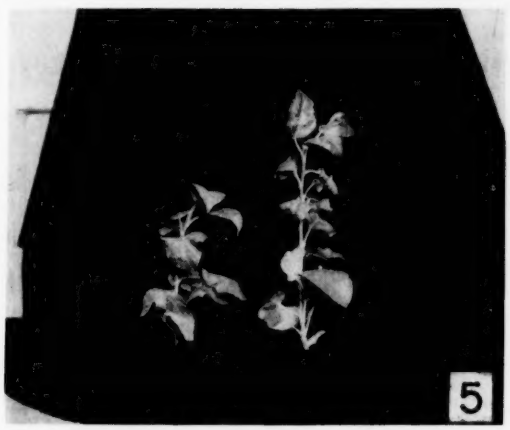
[2] Assistant Professor and Research Assistant, respectively, Department of Entomology, University of Wisconsin, Madison.



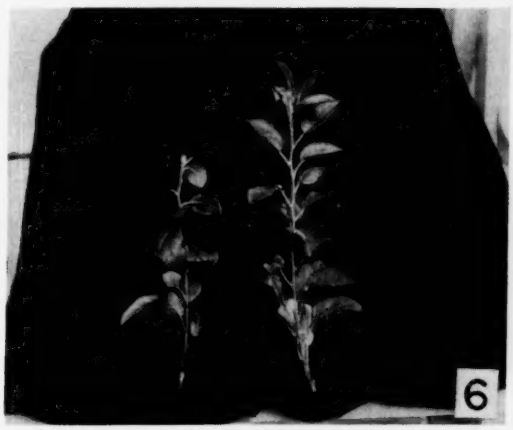


FIG. 1.—"Aphid Apples"—apple aphid injury to immature fruit.
FIG. 2.—Apple-aphid russeting and sooty mold fungus on mature apple.
FIG. 3.—Apple-aphid infested terminal.
FIG. 4.—Apple-aphid injury to McIntosh terminals.
FIG. 5.—Comparison of untreated check (left) and treated (right) Cortland terminal growth on young trees in apple-aphid studies.
FIG. 6.—Comparison of untreated check (left) and treated (right) Red Delicious terminal growth on young trees in apple-aphid studies.

Table 1.—The effect of two environments on the apple-aphid populations on two varieties in a young nonbearing apple orchard. Door County, Wisconsin. 1959.

Observation Date	Average Number of Aphids per Leaf[a]			
	Treated		Untreated	
	Red Delicious	Cortland	Red Delicious	Cortland
June 2	0.0	0.0	0.2	0.9
9	1.0	1.4	1.0	2.6
16	16.0	17.6	27.3	64.6
24	52.3	74.7	99.2	190.2
30	131.0	240.7	69.1	275.8
July 7	0.2[b]	2.7[b]	66.2	368.6
14	60.5	134.6	120.3	407.7
21	158.6	179.8	56.0	173.4
28	82.6	9.0	13.6	62.4
Aug. 4	82.6	15.4	25.0	61.1
11	5.1	0.8	22.6	2.8
18	0.1	0.0	5.9	0.0
25	0.0	0.0	0.0	0.0
Sept. 1	0.0	0.0	0.0	0.0

[a] Pretreatment count = 0.53 aphid/leaf on May 26.
[b] A systemic aphicide was applied July 2.

July 14 when a population peak of 264 per leaf was noted (table 2). The population declined steadily thereafter, finally disappearing the latter part of August. A similar increase was evident in the treated section through June 30 prior to the second aphicide spray on July 2. The recovery of the aphid population following this spray was probably due to reinfestation by alates as indicated by their numbers on July 14. The population on the treated trees quickly declined following a second peak on July 21 and disappeared from the foliage by the end of August. A few more aphids were present a week later on the treated than on the untreated trees.

The apple aphid also disappeared from bearing orchards in the area at approximately the same time and were not observed again on apple until July, 1960. The aphid was present on apple foliage throughout the growing seasons of 1956, 1957, and 1958. The reason for their disappearance in 1959 is not known. However, the weather records for the Peninsular Branch Station show that there was less rainfall and higher temperatures for June and July of 1959 than in any of the other years. This would seem to indicate that it was due primarily to physical factors. There were almost twice as many aphids per leaf on any given date in 1958 as compared with 1959. Gambrell & Gilmer (1960) recorded similar observations on the apple aphid population in New York for 1958 as compared with the previous 10 years. The weather records show that there was only one-half as much rainfall and an average of 5° F. higher temperature in June and July of 1959 as compared with 1958. The interaction of the two physical factors seemingly created conditions unfavorable to tree growth, and resulted in both a cessation of terminal growth and a hardening off of the leaves which apparently adversely affected aphid reproduction. An abnormal amount of rainfall in May and June 1960 was probably the principal reason for the absence of all aphids on apple foliage before July. Twice the normal amount of rain occurred during May when aphid eggs normally hatch.

The presence of alate forms was similar in the untreated check and treated environment through June 24. Although data were not taken, the highest number of alates probably occurred around July 1 as indicated by their numbers on June 24 and July 14. The time would coincide with the presence of the second generation which Matheson (1919) reported had the highest percentage of alates. The number of alates decreased abruptly following the population peak in both sections.

Parasitism was less than 0.1% and thus was considered a minor limiting factor.

The predator population increased concurrently with the aphid population, and reached a peak 1 week later on the check trees, and at the same time on the treated trees. The higher predator population in the treated section on July 21 resulted from an influx of adult coccinellids attracted by the higher aphid population. Adult coccinellids were the most abundant adult predators, and reached their highest numbers from July 14 to 28. The nine-spotted lady beetle, *Coccinella novemnotata* Herbst, was the most common species, with an average of 14/check tree by July 14. Coccinellid and syrphid fly adults were present May 26. Syrphid fly larvae were the most numerous immature predators feeding on aphids on the leaves. The first anthocorid nymph was recorded June 9; syrphid fly larvae June 16; coccinellid larvae June 24; and aphid lion larvae June 30. All predators were present by July 14. The highest number of predaceous larvae per leaf occurred July 21, being slightly more abundant in the treated than in the untreated environment.

The untreated trees were coated with honeydew and a sooty mold fungus, and had started to harden off by July 14, whereas treated trees were not covered and did not begin hardening off until a week later. The aphid population in both sections declined concurrently with the hard-

Table 2.—The effect of two environments on the bionomics of the apple aphid and its predators on young nonbearing apple trees. Door County, Wisconsin. 1959.

Observation Date	Average Number per Leaf[a]					
	Treated			Untreated		
	Aphids		Predators[b]	Aphids		Predators[b]
	Apterous	Alates		Apterous	Alates	
June 2	0.0	—	—	0.6	—	—
9	1.2	0.1	0.0	2.3	0.1	0.0
16	16.8	1.7	0.0	45.9	3.3	0.1
24	63.5	4.0	0.4	144.7	4.3	0.8
30	185.9	—	0.7	172.5	—	0.6
July 7	1.4	—	0.0	217.4	—	0.8
14	97.5	14.5	0.0	264.0[c]	1.4	0.8[d]
21	169.2[e]	7.6	2.3	114.7	0.4	1.9
28	45.8	0.1	0.8	38.0	0.1	0.5
Aug. 4	34.0	0.0	0.1	43.0	0.1	0.0
11	2.9	0.1	0.0	12.7	0.0	0.0
18	0.1	0.0	0.0	3.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0
Sept. 1	0.0	0.0	0.0	0.0	0.0	0.0

[a] Red Delicious and Cortland; Pre-treatment count = 0.53 aphids/leaf on May 26.
[b] Included syrphid fly, coccinellid, lacewing larvae, and anthocorid nymphs.
[c] Trees covered with honeydew; terminals hardening off.
[d] All predator species present; adult coccinellids numerous.
[e] Terminals just beginning to harden off.

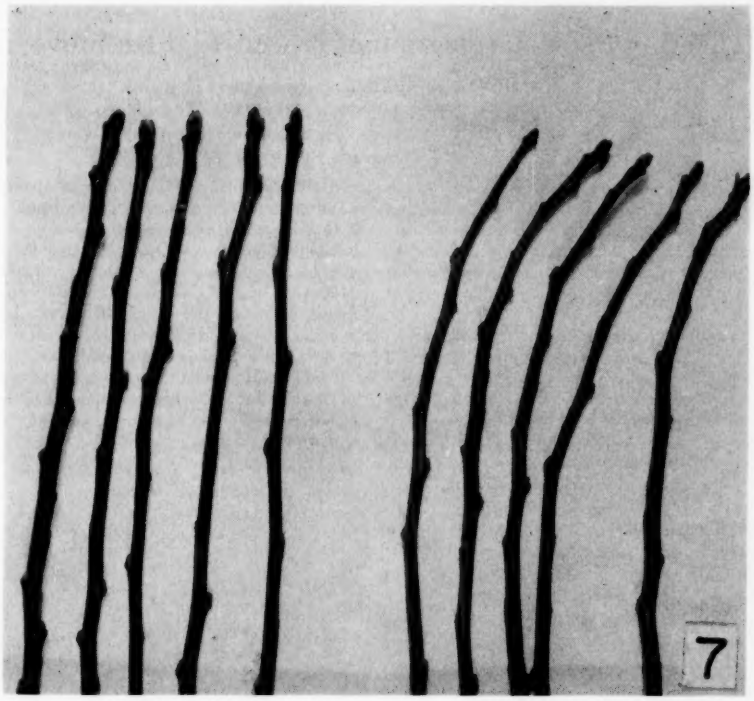


FIG. 7.—Comparison of Red Delicious terminals sampled at random from young trees during dormancy showing effect of high apple aphid population on the previous season's growth.

ening off of the foliage and cessation of terminal growth. The population thus apparently accelerated a condition unfavorable for further aphid reproduction.

Predators, host variety, and aphid abundance (intraspecific competition) were the primary biotic factors found affecting the apple aphid population. The latter was apparently the major limiting biotic factor.

The effect of the apple aphid on terminal growth is shown in table 3. Some of the more tender shoots were killed. The most common injury was the curling, stunting, and weakening of the terminals which increased the chance of winter mortality (figs. 5, 6 and 7). The increase in terminal growth on the treated trees was greater than on the untreated check. The differences were highly significant (1% level). The results were comparable with those obtained in preliminary studies in 1958 under higher aphid populations.

Table 3.—Effect of apple aphid on terminal growth of young nonbearing Red Delicious and Cortland trees in treated and untreated environment. Door County, Wisconsin. 1959.

MEAN TERMINAL GROWTH IN INCHES[a]			
Red Delicious		Cortland	
Untreated	Treated	Untreated	Treated
7.91	12.96[b]	9.90	11.38[b]

[a] Each value is a mean of five replicates each consisting of 10 subsamples.
[b] Significantly different from check at 1% level.

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Factors Affecting the Volatilization of Insecticidal Residues from Soils[1]

C. R. Harris[2] and E. P. Lichtenstein,
Department of Entomology, University of Wisconsin, Madison

Abstract

Laboratory and field studies showed that vapors toxic to both vinegar flies (*Drosophila melanogaster* Meigen) and house flies (*Musca domestica* L.) were given off from aldrin-, heptachlorphorate-, lindane-, heptachlorepoxide-, and dieldrin-treated soils. No evidence of volatilization was obtained from soils treated with DDT, Sevin® (1-naphthyl *N*-methylcarbamate), malathion, and parathion.

An increase in the rate of aldrin volatilization from the soil resulted from increases in: Insecticide concentration in the soil; soil moisture; relative humidity of air passing over the soil; soil temperature; and rate of air movement over the surface of the soil. A decrease in the rate of aldrin volatilization was noted in dry soils containing increasing amounts of clay and organic matter, and in wet soils containing increasing amounts of organic matter. The amount of soil in a given volume (bulk density) had no effect on the rate of aldrin volatilization.

In laboratory tests conducted over intervals of 5 to 7 days, it was possible to account for 90% to 96% of the aldrin initially applied to three soil types (Quartz sand, Plainfield sand and Miami silt loam). It was found that 55% to 80% of the initially applied aldrin was present as aldrin 5 to 7 days after application, while only small amounts were converted to dieldrin. From 16% to 38% of the applied aldrin volatilized from the soil. Initially, the rate of aldrin volatilization was rapid, but over longer periods of time the insecticide became "bound" to the soil and volatilization decreased 1 day after insecticidal application to a constant rate which varied with the soil type.

The persistence of insecticidal residues in soils has been investigated during recent years (Chisholm et al. 1951, Fleming et al. 1951, Ginsburg & Reed 1954, Lichtenstein et al. 1960). However, most of the data obtained have dealt with recoveries of the parent compounds from the soil, and in some cases with their metabolites such as dieldrin and heptachlorepoxide (Edwards et al. 1957, Gannon & Bigger 1958, Lichtenstein & Schulz 1960). It was found that within a relatively short period of time after application of an insecticide to the soil, a major portion of the insecticidal residue had "disappeared." It has often been assumed that these residues volatilize from the soil (Barthel et al. 1960, Getzin & Chapman 1960, Lichtenstein & Schulz 1961), and that vapors released from insecticide-treated soils cause insect mortality (Cook 1959, Harper 1958).

To obtain more information on the relative importance of volatilization in the disappearance of an insecticidal residue from the soil, investigations were conducted under both laboratory and field conditions to determine to what extent insecticides volatilize from soils, and on what factors this volatilization is dependent.

General Procedures

Bioassay Procedure.—Solutions of analytical grade insecticides dissolved in pentane were applied to the soil as described by Lichtenstein & Schulz (1959a). All calculations were based on the oven (50° C.) dry weight of the soil. Aliquots of treated soils were placed in galvanized iron containers (fig. 1), $3\frac{1}{4}$ inches in diameter and $6\frac{3}{4}$ inches high, and compacted to a volume of 60 ml. ($\frac{1}{2}$ inch in depth).

Cages for holding the test insects were made from salve tins, $3\frac{1}{8}$ inches in diameter and 2 inches in depth, from which the tops and bottoms were removed and replaced with 50-mesh copper screen. A planchet was welded on the inner surface of each cage to serve as a container for applesauce (food supply) (fig. 1). Fifty vinegar flies (*Drosophila melanogaster* Meigen) or 25 house flies (*Musca domestica* L.) were then counted into the cages. The cages containing the test insects were placed in the

Fig. 1.—Insect cages and galvanized iron containers used in determining the effects of toxic vapors released from insecticide-treated soils.

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[2] Formerly a graduate Research Assistant, University of Wisconsin and now Associate Entomologist, Entomology Laboratory, Chatham, Ontario.

galvanized iron containers on glass rods set 1½ inches above the surface of the treated soil. Hence, only vapors emanating from the soil could kill the test insects.

Duplicate cages containing the test insects were exposed above the insecticide-treated soils for varying periods of time. Mortality counts were made 24 hours after completion of the exposure period. Control mortality, if any, was corrected using Abbott's formula (Abbott 1925). For accurate measurements, time-mortality curves were constructed using 3-day-old vinegar flies. The average LT_{50} value obtained in two such assays was then used as a basis of comparison with other experiments. All bioassays were conducted at 23° ± 1° C. and 65% ± 5% relative humidity.

CHEMICAL PROCEDURES.—Soils treated with insecticides as described above were placed in a glass jar, 2½ inches in diameter and 5½ inches high, which was attached to a trap (fig. 2), containing a suitable solvent. Insecticide vapors emanating from the soil were picked up in humidified air passing over the soil and collected in the solvent contained within the trap. A flowmeter was used to measure the rate of air movement, which was maintained at a rate of 1.0 liters of air per minute per trap. With an exhaust manifold preceding the flowmeter, it was possible to set up tests in series of six: a solvent-treated (control) sample in triplicate and an insecticide-treated sample in triplicate (fig. 3). A known amount of insecticide was added to the trap of one of the controls in order to determine the efficiency of the analytical procedure. To obtain sufficient amounts of insecticide vapors for analysis, air was passed over the soil for a period of 6 hours, unless otherwise specified. Soil temperatures were maintained at 22° ± 1° C. by placing the "soil jars" in an oil bath, and the relative humidity of the air was maintained at 100% by first passing the air through water.

Insecticides were extracted from soils as described by Lichtenstein & Schulz (1959a). Both soil and vapor extracts were cleaned up as described by Lichtenstein et al. (1960). DDT samples were not cleaned up. Aldrin and dieldrin were analyzed according to the colorimetric methods of O'Donnell et al. (1954, 1955). DDT was analyzed according to the colorimetric method described by Schechter & Haller (1945). The aldrin-dieldrin analysis was sensitive to 2 micrograms, DDT to 10 micrograms. Known amounts of aldrin, dieldrin, or DDT added to the soil or to the vapor trap were recovered to the extent of 85% to 97%.

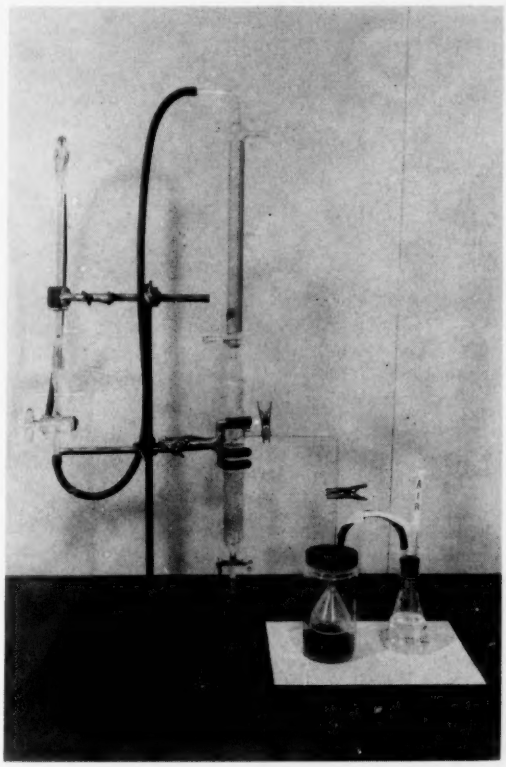

FIG. 2.—Apparatus used for collecting insecticidal vapors, released from treated soils, in a solvent trap.

SPECIFIC PROCEDURES

EVIDENCE OF VOLATILIZATION OF INSECTICIDAL RESIDUES FROM SOIL.—*a*) *Laboratory Tests.*—Aliquots of samples of dry Quartz sand treated at a rate of 4 p.p.m. with one of eight insecticides in pentane (lindane, aldrin, dieldrin, DDT, malathion, parathion, phorate) or acetone (Sevin® (1-naphthyl *N*-methylcarbamate)), or with equivalent amounts of solvent only, were placed in galvanized iron containers. Vinegar flies or house flies in screened cages were then placed above the treated sand for 6 or 24 hours. Mortality counts were made 24 hours after completion of the exposure period.

In another experiment, aliquots of dry Quartz sand, treated at a rate of 2 p.p.m. with either aldrin, heptachlor, heptachlorepoxide, or dieldrin, were placed in galvanized iron containers. Time-mortality curves using vinegar flies were then determined as well as the resulting fumigant LT_{50} values.

The rates of volatilization of aldrin, dieldrin, and DDT from dry Quartz sand were determined by chemical analyses. Aliquots of sand, treated with these insecticides at 4 p.p.m., were placed in "soil jars" (figures 2, 3). Aldrin and dieldrin vapors were collected in hexane for 6 hours immediately following soil treatment. DDT vapors were collected in benzene for 30 hours (6 hours per day for 5 days). All tests were conducted at 30° ± 1° C. The vapors collected were then analyzed colorimetrically for aldrin, dieldrin, or DDT as described above.

b) *Field Tests.*—Screened cages containing vinegar flies were exposed in the summer of 1959 above Carrington silt loam field plots treated 1 year previously (Lichtenstein 1960) with either aldrin or heptachlor at a rate of 25 pounds per acre. The cages containing the test insects were placed on glass rods at a distance of 0.25 to 0.5 inch or 1.25 to 1.5 inches above the soil surface. In some instances the cages containing the flies were covered with galvanized iron containers. Daytime temperatures caused high check mortalities. Therefore, the cages containing the flies were exposed in duplicate above the insecticide-treated soils for either 12 hours overnight or 24 hours over 2 nights. During the daytime, the cages containing

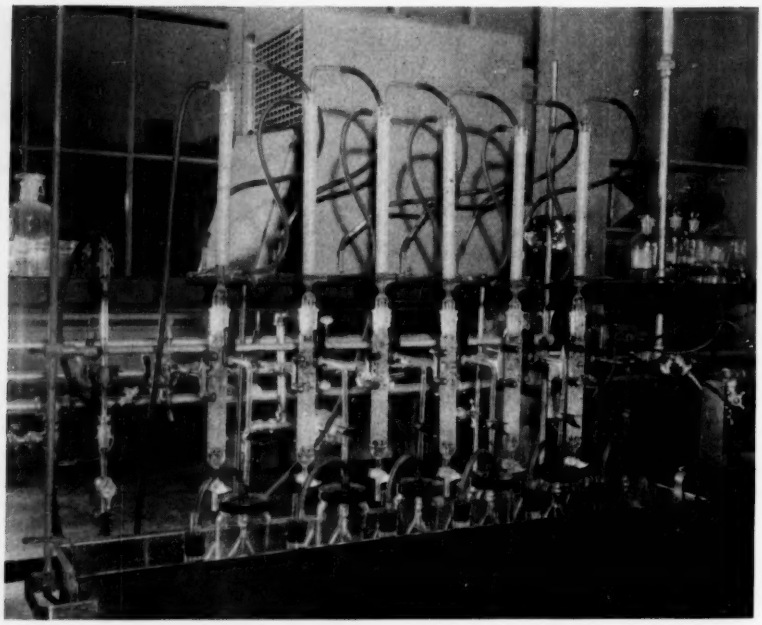

FIG. 3.—Vapor-collection apparatus in series.

the flies were held in an incubator at 23°±1° C. and 65%±5% relative humidity. Mortality counts were made 24 hours after completion of the final exposure period.

FACTORS AFFECTING THE VOLATILIZATION OF INSECTICIDAL RESIDUES FROM SOILS.—*a) Bulk Density of the Soil and Concentration of Insecticide in the Soil.*—Three experiments were conducted to determine the influence of bulk density (weight/volume) of the soil and concentration of insecticide in the soil on the rate of insecticide volatilization.

i) Three samples of field moist Miami silt loam (19.6% water), weighing 602, 679, and 753 grams respectively, were each treated with 2,000 micrograms of aldrin. Aliquots of these soil samples weighing 60.2, 67.9, and 75.3 grams were placed in galvanized iron containers and then compacted to the same volume of 60 ml., resulting in various weights of soil per unit volume. The concentration of insecticide in the soil decreased with increasing soil weight.

ii) A field moist Miami silt loam (23.8% water) was treated with aldrin at 4 p.p.m. Aliquots of the treated soils, of increasing weight, were placed in galvanized iron containers. The soils in all containers were then compacted to a volume of 50 ml. Though the bulk density of the soil was increased, the concentration of insecticide in the soil was constant.

iii) Samples of field moist Miami silt loam (24.1% water) were treated with aldrin at concentrations of 16.0, 8.0, 4.0, 2.0, 1.0, and 0.5 p.p.m. Aliquots of the treated samples (62.1 grams of wet soil) were then placed in galvanized iron containers and compacted to a volume of 60 ml.

Time-mortality bioassays were conducted in all three experiments (i, ii, iii) and fumigant LT_{50} values were determined.

b) Soil Type.—To determine the effect of soil type on the rate of insecticidal volatilization, six soils, either oven dry or wet (at field moisture capacity: the maximum percentage of water, based on the oven-dry weight of the soil that a well drained soil will hold), were treated with aldrin at 4 p.p.m. Aliquots of the treated dry and wet soils (representing identical dry weights of soil) were then placed in galvanized iron containers and compacted to a volume of 60 ml. Bioassays were conducted and fumigant LT_{50} values were obtained.

The characteristics of the soil types were also determined. Organic matter content of a particular soil type was obtained using the procedure described by Walkley & Black (1934). The analysis of the mineral fractions was conducted as described by Day (1956), after first oxidizing the organic matter from the soil with hydrogen peroxide.

c) Soil Moisture and Relative Humidity.—To determine the effect of soil moisture on the rate of insecticide volatilization from soils, seven samples of Miami silt loam containing 26.9%, 23.5%, 14.9%, 9.4%, 6.2%, 1.4%, and 0.0% water (oven dry) were treated with aldrin at 4 p.p.m. Aliquots of the treated soils were then placed in galvanized iron containers and bioassayed. Fumigant LT_{50} values were obtained from the resulting time-mortality curves.

The influence of relative humidity of the air passing over the soil on the rate of aldrin volatilization was also determined. Aliquots of wet and dry Quartz and Plainfield sands, treated with aldrin at 4 p.p.m., were placed in "soil jars" (fig. 2). Tests were then conducted in such a way that air of either low (38%±6%) or high (100%)

humidity was passed over the soils. Vapors emanating from the soil were collected in hexane and analyzed colorimetrically for aldrin.

d) Soil Temperature and Air Movement.—To test the effect of soil temperature and air movement over the soil surface on the rate of insecticide volatilization from soil, a Plainfield sand (6.3% water) was treated with aldrin at 4 p.p.m. Aliquots of the treated sand were placed in "soil jars" and held at either 10°±1°, 22°±1°, 30°±1°, or 36°±1° C., in oil or ice water baths. Air flow rates of 0.0, 0.5, and 1.5 liters of air per minute per trap were utilized when soils held at 22°±1° C. were tested. The air flow rate in the remaining experiments was 1.0 liters of air per minute. Vapors emanating from the soils were collected in hexane and analyzed for aldrin.

QUANTITATIVE DETERMINATIONS OF INSECTICIDAL RESIDUES IN SOILS AND IN VAPORS EMANATING FROM THESE SOILS.—The fate of aldrin in three soil types over intervals of 5 to 7 days was determined by chemical analyses. Aliquots of a dry Quartz sand, a wet Plainfield sand (6.3% water), and a wet Miami silt loam (25.3% water) treated with aldrin at 4 p.p.m., were placed in soil jars. Vapors emanating from the soils were collected in hexane for 6 hours per day over a period of 5 days in the case of the Plainfield sand and for 7 days when the Quartz sand and Miami silt loam were tested. The hexane containing the 6-hour vapor collections was analyzed for aldrin and dieldrin (Miami silt loam) or aldrin only (Quartz sand, Plainfield sand). In addition, soils were analyzed initially for aldrin residues (immediately after soil treatment) and for aldrin (Quartz sand, Plainfield sand, and Miami silt loam) and dieldrin (Plainfield sand, Miami silt loam) residues at the end of the experiment.

RESULTS AND DISCUSSION

EVIDENCE OF VOLATILIZATION OF INSECTICIDE RESIDUES FROM SOILS.—*a) Laboratory Tests.*—Though none of the insects came into direct contact with the insecticide-treated soil, considerable mortality was obtained with both vinegar flies and house flies exposed above soils treated with lindane, aldrin, phorate, and dieldrin (table 1). This demonstrated that the toxicants must have volatilized from the soil, and passed through the air into the cages holding the test insects, thus causing mortality.

Table 1.—Per cent mortality of *D. melanogaster* and *M. domestica* as caused by insecticide vapors released from dry Quartz sand treated at 4 p.p.m.

	Mortality (%)			
	D. melanogaster		*M. domestica*	
	Exposure Time (hours)			
Insecticide	6	24	6	24
Lindane	87±9	—	100	—
Aldrin	100	—	96±3	—
Dieldrin	0	100	0	41±5
DDT	0	0	0	0
Malathion	0	0	0	0
Parathion	0	0	0	0
Phorate	71±11	—	0	100
Sevin	0	0	0	0
Check	0	0	0	0

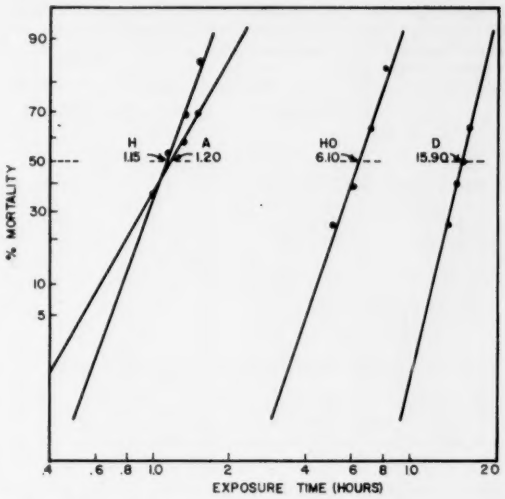

FIG. 4.—Time-mortality curves obtained by exposing *Drosophila melanogaster* to vapors of aldrin (A), dieldrin (D), heptachlor (H) and heptachlorepoxide (HO) emanating from Quartz sand treated at 2 p.p.m.

Aldrin and lindane vapors caused the highest mortality, followed by phorate. Dieldrin vapors caused the lowest mortality with house flies being less effected than vinegar flies. DDT, malathion, parathion and Sevin did not cause any fly mortalities, even after 24 hours' exposure above the treated sand. This lack of fumigant toxicity may have been because the insecticides were nonvolatile, or because the vapors given off were of low toxicity to the test insects.

Aldrin, dieldrin, heptachlor, and heptachlorepoxide commonly appear as residues in the soil. Therefore, more detailed information was obtained as to the comparative rates of volatilization of these materials when applied to dry Quartz sand at 2 p.p.m. (fig. 4). Fumigant LT_{50} values for aldrin and heptachlor were similar. However, the fumigant LT_{50} value for heptachlorepoxide was approximately five times larger than the values obtained when aldrin or heptachlor were used, while the fumigant LT_{50} for dieldrin was 13.5 times as large. If all the insecticides were equitoxic, the comparative rate of volatilization would be aldrin = heptachlor > heptachlorepoxide > dieldrin. However, contact bioassays with vinegar flies revealed that while aldrin and heptachlor were equitoxic, the LD_{50} values for heptachlorepoxide and dieldrin were 1.6 to 1.8 times larger than those obtained when the parent compounds were used. Nevertheless, reduced toxicity alone cannot account for the long periods of time required to cause 50% mortality when vinegar flies were exposed to vapors of heptachlorepoxide and dieldrin.

The data obtained by bioassay were confirmed to some extent by chemical analyses of vapors emanating from Quartz sand treated with aldrin, dieldrin, or DDT at 4 p.p.m. During a period of 6 hours immediately following treatment of the sand, 6.52%±0.12% of the applied aldrin, and 0.60%±0.26% of the applied dieldrin had volatilized. No DDT vapors could be detected even during a 30-hour period.

Table 2.—Per cent mortality of *D. melanogaster* caused by insecticide vapors released from Carrington silt loam field plots treated 1 year previously with aldrin and heptachlor at a rate of 25 lb./acre.

Treatment	Distance of Cage from Soil (inches)		
	1.25–1.5		0.25–0.5
	Covered[a]	Uncovered[b]	Uncovered[b]
Aldrin	64±24	8±2	52±2
Heptachlor	98±2	11±1	56±4
Control	0	0	0

[a] Exposed 12 hours overnight.
[b] Exposed 24 hours over 2 nights.

b) Field Tests.—The results of the tests on aldrin- and heptachlor-treated field plots (table 2) indicated that significantly higher mortalities were obtained under two conditions: (i) when the cages containing the vinegar flies were covered; and (ii) when cages left uncovered were placed closer to the soil surface. The results indicated that, while detectable amounts of insecticidal vapors were present above insecticide-treated field plots, these vapors were rapidly dissipated by air movement (wind.)

Factors Affecting the Volatilization of Insecticidal Residues from Soils.—*a) Bulk Density of the Soil and Concentration of Insecticide in the Soil.*—When the bulk density of the soil (weight/volume) was increased, while using a constant amount of aldrin, the rate of aldrin volatilization decreased (table 3, a). This was related to a decrease in insecticide concentration in the soil. However, when the bulk density of the soil, as well as the amount of insecticide applied to the soil, was increased (insecticide concentration constant), the rate of aldrin volatilization remained constant (table 3, b). Therefore, bulk density of the soil did not influence the rate of aldrin volatilization, whereas an increase in concentration of insecticide within the soil resulted in an increase in the rate of volatilization of the toxic residues.

After a Miami silt loam had been treated with aldrin at rates ranging from 0.5 to 16.0 p.p.m., it was found (fig. 5) that aldrin volatilized from the soil at a rate which was proportional to the concentration of aldrin within the soil.

b) Soil Type.—In both wet and dry soils aldrin vapors were released most rapidly from a Quartz sand and least rapidly from a muck (table 4). The rate of aldrin volatilization was much more rapid from wet as compared with dry soils (fig. 6).

Table 3.—Effect of bulk density (weight/volume) and insecticide concentration on the rate of toxic vapor release from an aldrin-treated field moist Miami silt loam.

Grams of Soil		Bulk Density	Aldrin		LT_{50} (hours)
Dry	Wet		μg.	P.p.m.	
A. Volume=60 ml.					
50.3	60.2	0.83	200	3.98	2.00±0.15
56.8	67.9	0.95	200	3.52	2.65±0.15
63.0	75.3	1.05	200	3.17	3.65±0.05
B. Volume=50 ml.					
40.0	49.5	0.80	160	4.00	1.46±0.14
50.0	61.9	1.00	200	4.00	1.68±0.08
60.0	74.3	1.20	240	4.00	1.58±0.08
70.0	86.7	1.40	280	4.00	1.55±0.01

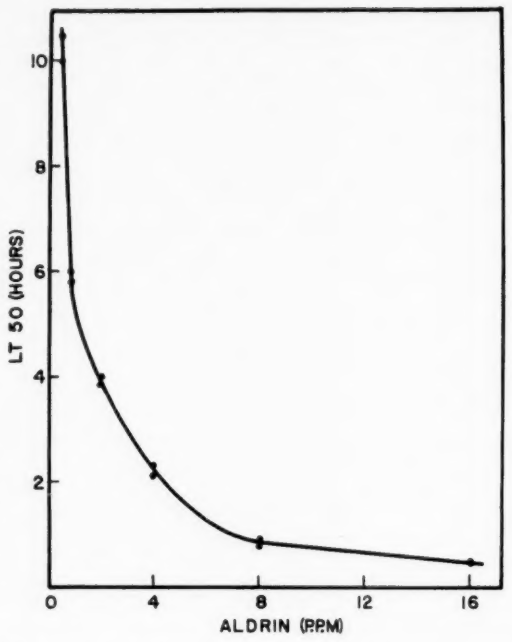

Fig. 5.—Effect of aldrin concentration in a wet Miami silt loam on the rate of aldrin volatilization.

There is no evidence that the elemental composition of the soil exerts any direct effect on the volatilization of an insecticidal residue (table 5). It also appears unlikely that cation exchange is a factor influencing insecticide persistence (Getzin & Chapman 1959). It seems most likely that an explanation of the results of this experiment may be obtained by considering the moisture, mineral, and organic contents of the soils tested.

It would appear that insecticides are strongly adsorbed to dry soils. Aldrin volatilized 37 times as rapidly from a wet Plainfield sand as from a dry sand (fig. 6). However, the differences in the LT_{50} values obtained with dry and wet soils became less pronounced as the organic matter content of the soils increased. Other authors (Barlow & Hadaway 1955, Lichtenstein & Schulz 1960, 1961) have reported that insecticides are strongly ad-

Table 4.—Fumigant LT_{50} of vapors released from 60 ml. of either ovendry or wet soils treated with aldrin at 4 p.p.m.

Soil Type	Water (%)	LT_{50} (hours) With Soils	
		Dry	Wet
Quartz sand	3.0	0.60±0.06	<0.25
Plainfield sand	6.6	22.25±0.25	0.63±0.01
Miami silt loam	26.6	51.25±0.25	1.68±0.08
Carrington silt loam	28.2	53.00±1.00	1.78±0.08
Muck I	81.8	83.50±1.00	6.00±0.15
Muck II	147.6	63.00±3.00	9.75±0.25

sorbed to dry soils and hence are extremely persistent, while in moist soils they are less strongly adsorbed and therefore less persistent.

The rate of aldrin volatilization from dry soils was not proportional to either the mineral or organic content of the soils (tables 4, 5). However, a general trend is apparent in that volatilization decreased with the increasing surface area within the soil, resulting from increasing amounts of clay and organic matter. With the addition of moisture to the soil, the adsorptive capacity of the mineral fraction of the soil apparently was satisfied by water molecules, while the nonpolar insecticide molecules were released. However, although the organic fraction of the soil also attracted water molecules, its adsorptive capacity was apparently not completely satisfied and some insecticide molecules were retained. Consequently in moist soils the rate of aldrin volatilization decreased with increasing organic matter.

c) Soil Moisture and Relative Humidity.—Fumigant LT_{50} values obtained with samples of Miami silt loam, treated with aldrin at 4 p.p.m., but containing varying amounts of water indicated that soil mositure had a pronounced effect on the rate of aldrin volatilization (fig. 7). The fumigant LT_{50} obtained with an ovendry treated soil was 55 times as large as that obtained with a soil containing 26.9% water. Increasing soil moisture apparently resulted in increasing volatilization of aldrin from the soil. However, the increasing relative humidity of the air above the soils, as tests progressed from dry to wet soils, may also have had some effect.

In a subsequent series of tests (table 6), the effect of both air humidity and soil moisture on the rate of insecticide volatilization was determined. In dry soils little or no volatilization occurred when the humidity in the air was low. However, when the air passing over a dry soil was humid, some volatilization of insecticide occurred. Apparently the insecticide molecules were displaced from the soil particles by water molecules in the air, resulting in an increase in the rate of volatilization. In wet soils, some volatilization occurred when the air passing over the surface of the soil was low in humidity. However, the surface of the soil became dry and the rate of volatilization decreased. Maximum insecticide volatilization occurred when humid air was passed over wet soil. Somewhat similar results were obtained by Barlow & Hadaway (1956).

d) Soil Temperature and Air Movement.—The data obtained in temperature studies showed that the rate of aldrin volatilization increased with increasing soil temperature. At 10° C., 2.04% ± 0.26% of the applied aldrin volatilized from the Plainfield sand during a period of 6 hours immediately following soil treatment. At 22° C., 4.88% ± 0.13% volatilized; at 30° C., 7.80% ± 0.19% volatilized; and at 36° C., 10.00% ± 0.59% volatilized.

Air movement was also found to influence the rate of aldrin volatilization. When no air was passed over the field moist Plainfield sand treated with aldrin at 4 p.p.m., only traces (<0.5%) of the applied aldrin volatilized from the soil during a period of 6 hours immediately following soil treatment. However, with air flow rates of 0.5 and 1.5 liters of air per minute, 4.39% ± 0.18% and

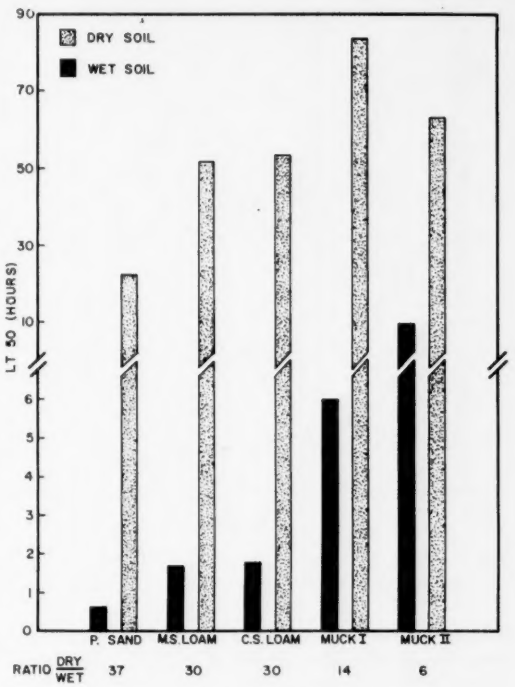

FIG. 6.—Effect of wet and dry soils on the rate of insecticide volatilization. (Soil treatment: 4 p.p.m. aldrin.)

Table 5.—Characteristics of soil types used in experiments.

Soil Type	Total N (%)	Pounds/Acre Available P	Pounds/Acre Available K	C.E.C.[a]	O.M.[b]	F.M.C.[c]	Per Cent Sand Particle Size (mm.) 2–.05	Per Cent Silt Particle Size (mm.) .05–.002	Per Cent Clay Particle Size (mm.) <.002
Quartz sand	—	—	—	—	0.0	—	100	0	0
Plainfield sand	0.72	23	55	4.3	0.8	6.8	91	3	6
Miami silt loam	0.11	128	200	25.5	3.6	26.0	5	76	19
Carrington silt loam	0.25	110	250	23.0	4.7	28.7	5	71	24
Muck I	1.33	340	900	88.0	31.9	80.0	13	75	12
Muck II	2.15	325	90	91.2	56.6	151.2			[d]

[a] Cation exchange capacity in milliequivalents per 100 g. ovendry soil.
[b] Per cent organic matter.
[c] Field moisture capacity based on the ovendry weight of the soil.
[d] A suitable mineral sample could not be obtained for analysis.

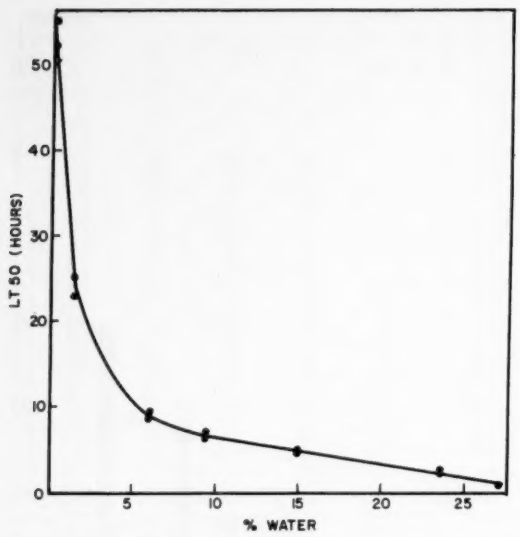

FIG. 7.—Effect of moisture content in a Miami silt loam on the volatilization of aldrin. (Soil treatment: aldrin 4 p.p.m.)

6.30%±0.36%, respectively, of the applied aldrin volatilized during a 6-hour period. The rate of insecticide volatilization from the soil is therefore dependent on the amount of air passing over the soil. Consequently, under field conditions, the rate of volatilization of an insecticidal residue from the soil may be retarded in areas of dense vegetative cover.

QUANTITATIVE DETERMINATION OF INSECTICIDAL RESIDUES IN SOILS AS WELL AS IN VAPORS EMANATING FROM THESE SOILS.—In the dry Quartz sand, over which air had been passed for 7 days, 6 hours daily, 55% of the applied aldrin was still present, while 38% had volatilized (table 7). No analysis for dieldrin was conducted on either the soil or air fractions, since it was assumed that no conversion of aldrin to dieldrin would occur during this period (Lichtenstein & Schulz 1961).

In the field moist Miami silt loam, 80% of the applied aldrin was recovered from the soil after 7 days while 16% had volatilized. No dieldrin vapors were found in the air passed over the soil (table 7). The dieldrin fraction from the soil was lost. However, based on available data (Lichtenstein & Schulz 1960), it was estimated that 3% to 4% of the applied aldrin would have been converted to dieldrin.

In tests conducted with a field moist Plainfield sand, 91.4% of the initially applied aldrin was accounted for after 5 days: 73% was recovered from the soil as aldrin; 2.4% was recovered from the soil in the form of dieldrin; and 16% of the applied dosage was recovered in the vapor form.

In all tests, more than 90% of the aldrin originally applied to the soil was accounted for. These figures seem to indicate that during the first 5 to 7 days after application, the disappearance of aldrin residues from the soil is due primarily to volatilization, while epoxidation occurs to a lesser extent. If the air had been moving over the soil for 24 hours rather than 6 hours per day, the amount of volatilization would probably have been much larger.

The amounts of aldrin emanating from the soils over periods of 5 to 7 days were largest during the first day (fig. 8). It will be noted that during the first 6 hours after treatment of the soil, the rate of volatilization was approximately the same regardless of soil type. During the second and third 6-hour intervals a slight reduction in the rate of volatilization occurred with the Quartz sand, and more marked reductions occurred with the Plainfield sand and the Miami silt loam. After this, aldrin volatilization was relatively constant, at a rate dependent on the soil type. It would appear that the insecticide becomes "bound" to the soil shortly after application. Consequently, it would be expected that under field conditions, a residue of a volatile insecticide such as aldrin will disappear relatively quickly immediately following application, but at a much slower rate over longer periods of time. Field investigations have shown similar results (Barthel et al. 1960, Lichtenstein & Schulz 1959b).

From all of these experiments, it is evident that volatilization of insecticidal residues from soil is a major factor in their "disappearance."

Table 6.—Effect of relative humidity of air on the volatilization of aldrin from wet and dry soils treated with aldrin at 4 p.p.m.[a]

SOIL TYPE	RECOVERY OF ALDRIN VAPORS DURING A 6-HOUR PERIOD IN PER CENT OF APPLIED	
	38%±6% R.H.	~100% R.H.
Quartz sand (dry)	traces (<0.50)	6.16±0.33
Quartz sand (wet[b])	3.99±0.29	7.33±0.64
Plainfield sand (dry)	0.00	1.28±0.05
Plainfield sand (wet[c])	2.19±0.09	4.52±0.16

[a] (Temperature = 22 °±1° C.; air flow = 1.0 liters/minute.)
[b] 3% water in soil.
[c] 6.3% water in soil.

Table 7.—Aldrin and dieldrin residues in soils and in air passed over these soils.[a]

RESIDUES FROM	IN PER CENT OF INITIALLY RECOVERED ALDRIN FROM SOILS	
	Quartz Sand	Miami Silt Loam
Soils[b]		
Aldrin	55.3	79.7
Dieldrin	—	Lost
Vapors[c]		
Aldrin	37.9	16.3
Dieldrin	—	0.0
Total recovery	93.2	96.0

[a] (Treatment: Aldrin @ 4 p.p.m.; temperature = 22° ± 1°C.; relative humidity = ~100%; air flow = 1.0 liter/minute.)
[b] Seven days after treatment.
[c] Collected for 6 hours per day, for 7 days.

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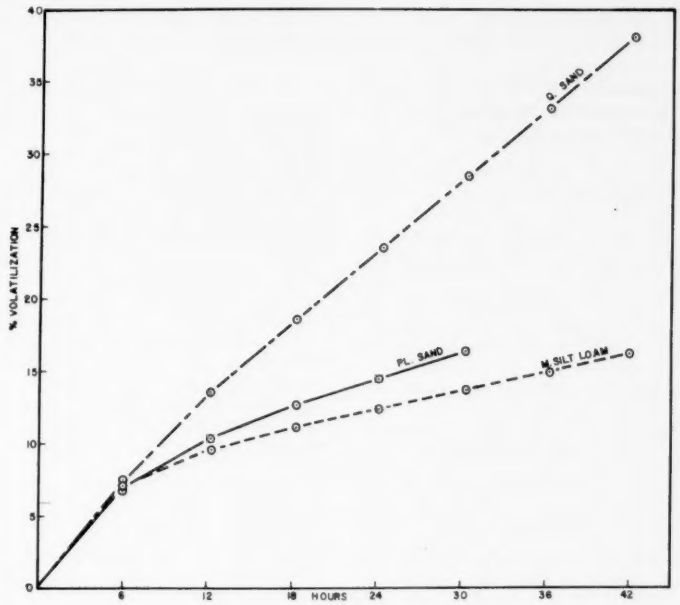

FIG. 8.—Amounts of aldrin vapors emanating from three soil types in per cent of aldrin initially recovered from the soils. (Soil treatment: 4 p.p.m.)

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SCIENTIFIC NOTES

The Primary Evaluation of Three Organophosphorus Compounds for Possible Use in the Control of Livestock Insects[1]

O. H. Graham, *Livestock Insects Investigations, Entomology Research Division, Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

In July 1957, the General Chemical Division of Allied Chemical and Dye Corporation submitted samples of three candidate insecticides to the Livestock Insects Laboratory at Kerrville, Texas for evaluation in their screening test program. These compounds were coded as General Chemical 3582, 3583, and 4072. Their structure was held in confidence at that time, but they have since been identified as follows: General Chemical 3582 (1-(2,5-dichlorophenyl)-2,2-dichlorovinyl diethyl phosphate); 3583 (2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate); and 4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate).

The unpublished results of screening tests conducted by H. G. Alford[2] indicated that all three compounds were highly active as screw-worm larvicides and stable fly toxicants. Full-grown screw-worm larvae were killed by each of the compounds at concentrations as low as 0.025%, which indicated they were about four times as toxic as lindane. An evaluation of the residual effect of the materials as toxicants for adult stable flies indicated that General Chemical 3582 and 3583 were effective for 17 weeks and 4072 for 25 weeks. In comparison, DDT and methoxychlor in the same test were effective for 40 or more weeks.

The results of animal systemic screening tests with General Chemical 4072 have been published by Drummond (1960), but the results of his tests with the other two compounds have not. Oral and subcutaneous doses of General Chemical 3582 and 3583 given to guinea pigs did not kill screw-worms (*Callitroga hominivorax* (Coquerel)), stable flies (*Stomoxys calcitrans* (L.)), or lone star ticks (*Amblyomma americanum* (L.)) that fed on these animals. However, subcutaneous dosages as low as 10 mg./kg. of General Chemical 4072 to guinea pigs did cause 100% kill of screw-worms and stable flies.

Of the three compounds, General Chemical 3582 appeared to be the most toxic to warm-blooded animals, since it killed guinea pigs given 50 mg./kg. orally and 25 mg./kg. subcutaneously. General Chemical 3583 killed guinea pigs given 100 mg./kg. orally and subcutaneously, but not those that received 50 mg./kg. Guinea pigs were killed by 50 mg./kg. of General Chemical 4072 given subcutaneously, but survived subcutaneous doses of 25 mg./kg. as well as oral doses of 100 mg./kg. A very rough approximation of their toxicity, therefore, was that they were in the same general class with butonate and DDVP, slightly less toxic than Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), and slightly more toxic than Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate).

In a recent publication, Mulla (1960) rated General Chemical 4072 as 1½ to 2 times more toxic to eye gnats (*Hippelates collusor* (Townsend)) than 3582 and approximately four times as effective as 3583. The initial toxicity rather than the residual effect of the chemicals was evaluated by the results of his laboratory screening tests.

These primary evaluations indicated that the three compounds offered considerable promise as effective insecticides for the control of livestock insects. As will be shown by the eight subsequent papers in this series, two of the three compounds have been used as spray applications on several classes of livestock in both

[1] Accepted for publication July 12, 1961.
[2] Formerly of the Kerrville laboratory.

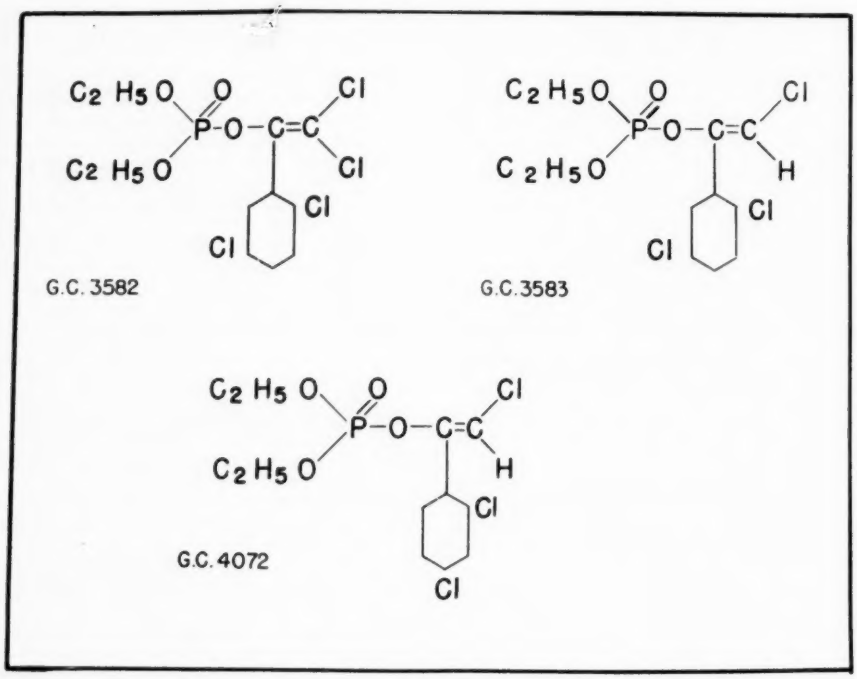

FIG. 1.—The molecular configurations of General Chemical 3582, 3583, and 4072.

laboratory and field tests. Using a cooperative approach, entomologists, chemists, and veterinarians have studied these compounds in an attempt to determine their entomological effectiveness, toxicity to livestock, and residues in meat and milk.

The identity of the last of the three compounds was released from confidential status in 1960. The molecular configurations are given in figure 1. It can be seen that General Chemical 3582 differs from 3583 in that it is a dichlorovinyl rather than a monochlorovinyl compound, and 3583 differs from 4072 only in the position at which one of the chlorine atoms is attached to the phenyl radical. Our preliminary tests indicated that all three compounds were biologically active, but that there were differences in their behavior. As will be reported in the following seven papers, these differences were even more apparent in the more advanced testing undertaken during 1958, 1959, and 1960.

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Tests with Sprays of General Chemical 4072 for Systemic Control of Cattle Grubs[1]

R. O. DRUMMOND, *Livestock Insects Investigations, Entomology Research Division, Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

General Chemical 4072 (2,chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) was reported by Drummond (1960) to be active as an animal systemic insecticide in preliminary laboratory screening tests. In initial tests with small numbers of cattle to determine its systemic effectiveness against cattle grubs (*Hypoderma* spp.), an oral dosage of 20 mg./kg. and a 0.5% spray were 68% to 79% effective in preventing grubs from reaching the cattles' backs. During the summer of 1960 a small test was conducted to determine if grub control could be obtained when sprays of 4072 were used to control other parasites of cattle. This paper presents the results of that test.

In a cooperative research program of the Entomology Research Division and the Animal Disease and Parasite Research Division, a small herd of Hereford cattle has been maintained on pasture near Kerrville, Texas. Each month the animals are examined for ectoparasites, and when ticks or flies become numerous, the animals are sprayed with 0.5% toxaphene in accordance with local ranch practice.

During the summer and fall of 1960, it was necessary to spray this test herd twice. On August 3, five yearlings and nine dry cows were sprayed with 0.25% General Chemical 4072 at the rate of 1 gallon per animal instead of with toxaphene. On September 1, the five yearlings were again sprayed with 0.25% Gen. Chem. 4072. The sprays were prepared from a wettable powder containing 25% active ingredient. On these same dates, the remainder of the herd—23 cows, 6 yearlings, and 14 calves—was sprayed with 0.5% toxaphene.

Table 1.—Results of tests with sprays of 0.25% General Chemical 4072 for systemic control of cattle grubs, 1960.

DATE SPRAYED	NUMBER AND KIND OF CATTLE	NUMBER INFESTED	AVERAGE NUMBER OF GRUBS	PER CENT CONTROL
8/3, 9/1	5 yearlings	1	0.80	94
8/3	9 cows	1	0.22	95
Untreated	6 yearlings	6	13.33	—
	23 cows	17	3.82	—
	14 calves	12	12.25	—

The backs of the animals were inspected for common cattle grubs (*Hypoderma lineatum* (de Villers)) at the regular monthly examinations, and cumulative counts of grubs for each animal were made by plotting the appearance of each grub on an outline map of the animal's back. The results of these examinations are summarized in table 1.

From these data, it is apparent that both one and two sprayings with 0.25% Gen. Chem. 4072 afforded a high degree of grub control. However, large-scale field tests will be needed to substantiate the data. It is interesting that systemic control of cattle grubs was achieved incidental to the control of other livestock pests.

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[1] Accepted for publication July 12, 1961.

Control of Horn Flies and Stable Flies with Three General Chemical Compounds[1]

R. H. ROBERTS, M. J. WRICH, R. A. HOFFMAN, and C. M. JONES, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

Since promising results were obtained in primary evaluation tests with General Chemical compounds 3582, 3583, and 4072, their entomological effectiveness was further tested in laboratory and field experiments. This paper presents the data that have accumulated from their use for several seasons in the control of horn flies (*Haematobia irritans* (L.)) and stable flies (*Stomoxys calcitrans* (L.)).

METHODS.—Before these materials were applied to an entire animal, they were evaluated by the spot-test method (Roberts et al. 1960) and then referred to veterinarians of the Animal Disease and Parasite Research Division for toxicological evaluation. After the toxicity data were available, additional spot tests were conducted to determine more exactly the entomological effectiveness of the safe levels. Concentrations that appeared to be both safe and effective were used in large-cage tests (Roberts et al. 1960).

These tests were conducted by spraying Jersey cattle with the candidate insecticides and exposing the treated animals to stable fly and horn fly attack at various intervals after treatment. Stable fly control was calculated by adding the difference between the number collected and number released to the number dead at 24 hours. Horn fly control was based on the length of time required to kill all the flies released on the treated animal; the treatment was considered to have failed if any of the released flies were alive after 24 hours.

In addition to the trials with Jerseys, groups of four yearling Herefords were sprayed with 0.5%, 0.25%, and 0.1% General Chemical 4072 and used in other large-cage tests. Since the toxicity of these concentrations to the cattle was being observed by the cooperating veterinarians, 2 to 2½ gallons of spray per animal were applied. One treated animal from each group was selected for exposure to the flies at any given test interval, but at each interval a different animal was used.

[1] Chemical names for these compounds are as follows:
3582 (1-(2,5-dichlorophenyl)-2,2-dichlorovinyl diethyl phosphate)
3583 (2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate)
4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate)
Accepted for publication July 12, 1961.

Field experiments were conducted at cooperators' ranches in Texas, Mississippi, and Nebraska. In Texas, cattle treated for horn fly control were sprayed to the point of runoff, usually about 1 gallon per head. In Mississippi and Nebraska, cattle treated for horn fly control were sprayed at the rate of 2 quarts per head; for stable fly control in Nebraska a dosage of 2 to 3 quarts per head was used.

In addition to the animal treatments, tests on premise control of stable flies were conducted at four locations in Nebraska. At three locations, applications to stable fly resting places were made at the rate of 100 mg. per square foot, and at one location at 150 mg. per square foot.

RESULTS.—In the first series of spot tests, all three compounds at 0.5% were superior to the standard, 0.5% methoxychlor. In these tests methoxychlor was effective for 8 days, General Chemical 3582 for 17 days, 3583 for 14 days, and 4072 for 20 days. Initial toxicological evaluation indicated that 3582 would be safe below 0.25% and 3583 at less than 0.1%. General Chemical 4072, which appeared safe at 0.5% in preliminary studies, was later found to be safe only up to 0.25% (Radeleff et al. 1961). Additional spot tests at lower concentrations indicated that 3582 was entomologically effective for 18 days at 0.1%, whereas 3583 at 0.05% was effective for only 1 day.

The data from large-cage tests with General Chemical 3582 and 4072 showed that both materials were superior to the methoxychlor standard for stable fly control. Methoxychlor at 0.5% gave more than 90% control for a 3- to 4-day period. General Chemical 3582 at 0.1% was equally effective for 6 days, and 4072 for 6 to 8 days at 0.5%, and 5 to 6 days at 0.1%. Horn flies were controlled for 18 days with 4072 at 0.5%, as compared with 14 days with methoxychlor.

Results of large-cage tests with the Herefords treated with General Chemical 4072 were affected by exposure of the animals to a total of 2¼ inches of rain between the 5th and 12th day after treatment. Stable fly control 5 days after treatment was 90%, 100%, and 86% at the concentrations of 0.5%, 0.25%, and 0.1%, respectively, but dropped to 18%, 24%, and 27% on the eighth day after treatment. One of the animals in the 0.5% group was poisoned and required atropine therapy (Radeleff et al. 1961).

The horn fly field tests (table 1) in Texas, Mississippi, and Nebraska indicated that wettable-powder formulations of 3582 and 4072 were equal to, or exceeded, the methoxychlor standard in residual effectiveness.

Control of stable flies with 4072 by premise treatment in Nebraska at rates of 100 and 150 mg. per square foot compared favorably with a methoxychlor treatment at 200 mg. per square foot. The number of stable flies per square foot was reduced from 8 to 15 before application to 1.5 or less for methoxychlor and 3.0 or less for 4072 for 30 or more days. No significant difference was noted between the 100- and 150-mg. application rates.

The control of stable flies in an isolated area in Nebraska after animal treatment with 4072 was estimated by the number of stable flies observed per square foot of fly-resting surface in a barn frequented by the treated animals. Prior to treatment, there was an average of 4.3 stable flies per square foot, and 5 to 10 flies per leg were noted on the animals. Three hours after treatment the animals were free of flies. Control continued to be excellent for 4 days, as indicated by low numbers on the animals and 0.5 per square foot on the resting surfaces 4 days after treatment. The number of flies on the resting surfaces increased by the seventh day to 1.1 per square foot. On the ninth day the average number dropped to 0.6, and the effectiveness of the material was further demonstrated by the fact that of 67 engorged flies collected from the barn and held in the laboratory, 66 were dead within 24 hours. The test was discontinued on the 11th day when a group of untreated animals was brought into the area.

In another Nebraska stable fly test, the treatment of 43 cattle did not reduce the stable fly population as determined by the number of flies per leg. However, at one location where the treated animals frequented a building with a concrete floor that was cleaned daily, dead flies were collected from the floor through the eighth day after treatment.

Table 1.—Horn fly field tests with General Chemical 3582 and 4072 compared with a methoxychlor standard spray.

Insecticide and Formulation	Concentration (%)	Number of Animals Treated	Number of Days Treatment Effective[a]
Kerrville, Texas (approximately 1 gal./head)			
Gen. Chem. 3582			
25% WP	0.1	107	22
50% EC	.1	41	33
Gen. Chem. 4072			
25% WP	.1	43	14
	.25	222	13
	.5	18	32
		21	22[b]
50% EC	.5	23	11
Methoxychlor			
50% WP	.5	336	21
Stoneville, Miss. (2 qt./head)			
Gen. Chem. 4072			
25% WP	0.25	65	10
		50	21
		60	7–14
		83	7
		65	14–21
	.5	19	14
		13	25
	.75	14	25
Methoxychlor			
50% WP	.5	75	12
Lincoln, Nebr. (2 qt./head)			
Gen. Chem. 4072			
25% WP	0.5	13	25
Methoxychlor			
50% WP	.5	8	21

[a] Treatment considered effective until the population reached or exceeded an average of 25 flies per head.

[b] Weather prevented later examination; at indicated days animals averaged 5 flies per head whereas untreated cattle in vicinity averaged 75 flies per head.

DISCUSSION.—From results obtained in the spot tests at a concentration of 0.5%, General Chemical 4072 was shown to have a longer residual effectiveness than 3582, which in turn was effective longer than 3583. At the lower concentration of 0.1%, 3582 was 2.5 times as effective as 4072. At 0.05%, 3582 and 4072 were equally effective, and both were seven times as effective as 3583.

In the horn fly field tests, 3582 at 0.1% protected the cattle for an average of 24 days, as compared with 21 days for the standard, 0.5% methoxychlor. Suspensions of 4072 at 0.5% protected cattle at least 7 days longer than the methoxychlor standard. Concentrations of 0.25% and 0.1% were less effective than the standard in tests conducted in Texas, but in Mississippi control with the 0.25% treatment was effective 4 days longer than the standard.

These preliminary data indicate that General Chemical 3582 and 4072 may be useful for the control of stable flies and horn flies at concentrations of 0.1% and 0.25%, respectively, but their value to the livestock producer will depend considerably on their effectiveness in controlling a greater variety of biting and nonbiting pests. Apparently, General Chemical 3583 is not safe for application to cattle at entomologically effective levels.

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Toxicity of General Chemical Compounds 3582, 3583, and 4072 to Screw-Worms in Laboratory and Field Tests[1]

M. J. WRICH,[2] W. F. CHAMBERLAIN,[2] and C. L. SMITH,[3]
Agric. Res. Serv., U.S.D.A., Kerrville, Texas

From 1957 through 1960 approximately 300 chemicals were tested against screw-worms (*Callitroga hominivorax* (Coquerel)) in vitro and in artificially infested sheep and cattle at the Kerrville, Texas, laboratory. Some of the more toxic larvicides tested under field conditions have been reported by Graham et al. (1959) and Wrich & Bushland (1960). This paper presents the results of laboratory and field screw-worm tests conducted from 1957 through 1960 with General Chemical compounds 3582, 3583, and 4072.

BEAKER TESTS.—The compounds were first screened by the beaker-test method described by Eddy & Graham (1950). The following tabulation presents the results of these tests against screw-worm larvae with the three compounds:

Gen. Chem. Compound	*P.P M. Causing Indicated Mortality at 48 Hours*	
	Apparent Zero	100%
3582	0.25	1.0
3583	1.0	—
4072	0.1	0.25

General Chemical 4072 was toxic to larvae in concentrations as low as 0.25 p.p.m., 3583 was ineffective at 1.0 p.p.m., and 3582 was effective only at 1.0 p.p.m. An exception occurred: One of the duplicate treatments of 3582 at 0 5 p.p.m. was toxic to larvae.

TESTS WITH ARTIFICIALLY INFESTED SHEEP.—Forty sheep, artificially infested with screw-worms under controlled conditions, were divided into 13 groups and sprayed or smeared with one of the test compounds. On each treatment date additional sheep were sprayed with 0.25% Co-Ral[4] (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) or smeared with EQ-335 for a standard of comparison. At the time of treatment, all except two sheep had one wound containing approximately 200 4-day-old larvae in the muscle of one of the hind legs; two sheep had a wound in each hind leg. For spray applications, a small power sprayer was used to apply about 200 ml. of insecticide to the wound and a surrounding area about 6 inches in diameter. A 1-inch paint brush was used to apply smear treatments to the wound and the area immediately surrounding it. The amount of smear applied varied according to the size of the wound, but all wounds were thoroughly treated.

The wounds were examined 24 hours posttreatment. If live larvae were present, the treatment was usually considered a failure and the compound not included in further tests. Effective treatments were challenged three times each week by exposing the sheep to gravid female flies in a screened cage until each wound had one egg mass containing about 200 to 300 eggs. Oviposition on moist wounds was rapid but difficult to induce on wounds that were dry and healing.

The results of eight tests are summarized in table 1. Four animals were smeared with General Chemical 3582; three of these healed, and one died 13 days after treatment, possibly of a bacterial infection. General Chemical 4072 was sprayed on two sheep; one healed and the other became reinfested. The wounds on four sheep were treated with this compound formulated as a smear; although two became reinfested, the smear was equal to EQ-335 in protective value.

Several concentrations of General Chemical 3583 were evaluated as sprays and found to be effective against the larvae infesting the wounds at the time of application. Ten of the 22 treated wounds healed in an average of 34 days, as compared with 30 days for animals sprayed with 0.5% Co-Ral. Smear formulations of the chemical compared favorably with EQ-335, but were slightly inferior to the Co-Ral spray. Even though General Chemical 3583 appeared to be as effective as EQ-335 and nearly as effective as Co-Ral, this compound was not field tested because it was reported by Animal Disease and Parasite Research Division veterinarians to be more toxic to the experimental animals than 4072.

TESTS WITH ARTIFICIALLY INFESTED CATTLE.—The effectiveness of General Chemical 4072 spray as a screw-worm toxicant and repellent was further tested on one Hereford steer. This animal was wounded and infested in one exposed and one hidden location each day for 4 successive days, so that at the time of treatment the larvae in the eight wounds ranged in age from 1 to 4 days. Five quarts of a 0.25% concentration of the insecticide, applied with a power sprayer, were used to wet the animal thoroughly. This treatment killed all larvae.

The residual effectiveness of the material was determined at 24-hour intervals by scraping the exudate and debris from a ½-inch circle in each wound and implanting about 200 newly hatched larvae. This procedure was continued until all wounds became reinfested or healed; no wound was challenged after it had become reinfested. Two of the challenged wounds became reinfested at 2 days after spraying, one at 4 days, four at 5 days, and one at 6 days.

FIELD TESTS.—Because tests with artificially infested sheep indicated that General Chemical 4072 sprays were more effective than EQ-335 smears for screw-worm control, and because other researches were obtaining favorable data with this chemical in their experiments, a decision was made to test 4072 under field conditions with a larger number of animals.

In September and October 1960, 395 sheep and goats were sprayed on four ranches near Harper and Mountain Home, Texas. Most of the test animals were freshly shorn and had shear cuts that made them susceptible to screw-worm attack. The animals at each of three ranches were divided into two groups; one group was treated with the test insecticide at 0.25% and the other with 0.25% Co-Ral as a control treatment. At one ranch, near Harper, General Chemical 4072 was the sole treatment, and the results of this test were compared with those obtained at the other three ranches.

Following treatment, the two groups were returned to adjacent pastures to subject the animals to uniform infestation pressures. In some instances, however, both groups were returned to the same pasture. When both groups were pastured together, the treated animals were allowed to dry thoroughly and then paint-branded for identification. The cooperating ranchers examined their animals periodically after spraying and recorded the numbers of infested animals.

All of the animals at three of the four ranches were protected from infestation throughout the 35-day test period. At the fourth ranch, near Harper, 9 infestations were recorded in the Co-Ral group; 3 of these were recorded at 7 and 9 days posttreatment. Six additional infestations were recorded between 24 and 30 days posttreatment. General Chemical 4072 was effective through 12 days posttreatment; 1 infestation was recorded on the 13th day.

[1] Chemical names for these compounds are as follows:
3582 (1-(2,5-dichlorophenyl)-2,2-dichlorovinyl diethyl phosphate)
3583 (2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate)
4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate)
Accepted for publication July 12, 1961.
[2] Livestock Insects Investigations, Entomology Research Division.
[3] Animal Disease Eradication Division.

Table 1.—A comparison of three General Chemical compounds with two standard treatments for the control of screwworms in artificially infested sheep, 1957–1960.

Insecticide	Concentration and Formulation	Number of Sheep					Average Number of Days for Wound to—	
		Treated	With 100% Kill of 4-Day-Old Larvae	Reinfested	Healed	Died	Become Reinfested	Heal
3582	1% smear	4	4	0	3	1	—	38
3583	1% smear	4	4	4			8	—
	3% smear	4	4	1	2	1	10	36
	0.1% spray	2	2	2			9	—
		4	4	3	1		11	48
	0.5% spray	4	4	1	3		27	33
		4	4	3	1		10	31
		2	2	1	1		8	21
	1.0% spray	4	4	1	3		5	25
		2	2	1	1		38	43
4072	1% smear	1	1	1			16	
		3	3	1	2		8	29
	0.25% spray	2	2[a]	1	1		2	14
Co-Ral	0.5% spray	8	8	5	3		11	30
EQ-335	3% smear	28	20	10	10		8	27

[a] On one animal two larvae, noticeably affected by the chemical, were alive at 24 hours posttreatment and dead at 48 hours. This animal remained in test.

Four other infestations in this group were recorded at 35 days posttreatment.

DISCUSSION.—At the conclusion of the field tests, the ranchers in the Harper area reported that, in their opinion, screw-worm infestation pressures had been light during the experiment. The rancher at Mountain Home classified screw-worm activity on his ranch at the time of the test as extremely light. The ranchers based their estimates on the number of screw-worm infestations that appeared in untreated livestock maintained on the ranches in accordance with regular ranch practices. It is not uncommon for these ranchers to treat 3 to 6 infestations a day during a season classified as having moderate screw-worm activity. It appears, therefore, that further field tests with General Chemical 4072 should be conducted in order to substantiate the results of this series of tests.

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Tests with General Chemical 3582 and 4072 for the Control of Ticks Affecting Livestock[1]

R. O. DRUMMOND, *Livestock Insects Investigations, Entomology Research Division, Agric. Res. Serv., U.S.D.A., Kerrville, Texas*

For the control of ticks on beef cattle the Entomology Research Division (1961) recommends sprays or dips containing 0.025% lindane, 0.15% Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)) or 0.5% toxaphene, dips of 0.25% and sprays of 0.5% Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), and sprays of 0.5% malathion and 0.75% ronnel. At the Livestock Insects Laboratory, new insecticides are compared with these recommended materials in field tests against lone star ticks (*Amblyomma americanum* (L.)) and winter ticks (*Dermacentor albipictus* (Packard)) on cattle. This paper presents results of tests against both species with sprays of General Chemical 3582 from a 50% emulsifiable concentrate and General Chemical 4072 from a 25% wettable powder.

LONE STAR TICKS.—In the vicinity of Kerrville, the lone star tick, a three-host species, is found on cattle from February through July. Livestock are subject to reinfestation by adults throughout this period, with the greatest numbers found from late May to early June. The number of ticks decreases rapidly with hot, dry, summer weather. A single spraying of 0.5% toxaphene will usually effect an adequate reduction in tick populations for 2 weeks.

The methods used in these tests were similar to those of Drummond et al. (1960). Numbers of adult ticks on certain areas of cows were recorded before treatment, the day after treatment, and weekly thereafter. At each of several ranches, one or more groups of five animals were sprayed with the candidate insecticides, and one group with 0.5% toxaphene from a 61% emulsifiable concentrate as a standard of comparison. After spraying, all treated cows were turned into the same pasture to insure equal exposure to ticks. Control was determined by comparing the numbers of ticks recorded posttreatment with those recorded before treatment. None of the animals were left untreated. Because of large numbers of ticks, cattle were sprayed three or four times during the tick season. The results of these tests are summarized in table 1.

General Chemical 3582 at 0.1% afforded excellent initial kill but was somewhat less effective than the standard 0.5% toxaphene at 1 and 2 weeks posttreatment. General Chemical 4072 at 0.5% was very effective at 1 day and 1 week, and was slightly more effective than 0.5% toxaphene in minimizing tick populations on the cattle through 4 weeks posttreatment. In a single test little difference appeared to exist between the effectiveness

[1] Chemical names for these compounds are as follows:
3582 (1-(2,5-dichlorophenyl)-2,2-dichlorovinyl diethyl phosphate)
4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate)

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Table 1.—Effectiveness of General Chemical 3582 and 4072 as sprays against lone star ticks on cattle, 1960.

Insecticide	Concentration (%)	Average Number of Adult Ticks on 5 Cows[a]					
		Before Treatment	After Treatment				
			1 Day	1 Week	2 Weeks	3 Weeks	4 Weeks
		Sprayed April 7					
4072	0.5	52.0	0.4	1.2	16.2	24.2	22.6
Toxaphene	.5	41.4	4.0	9.4	29.6	33.6	45.6
		Sprayed May 26					
3582	0.1	31.2	1.2	5.6	24.6	11.2	—
Toxaphene	.5	23.4	2.6	3.6	16.0	11.0	—
		Sprayed May 23					
3582	0.1	16.2	0.4	5.4	10.4	12.4	—
Toxaphene	.5	11.0	1.0	3.0	8.6	13.0	—
		Sprayed July 7					
4072	0.25	26.4	1.8	9.5	23.2	—	—
	.1	24.2	0.8	9.8	31.8	—	—
Toxaphene	.5	19.0	2.0	6.8	27.0	—	—

[a] On portions of dewlap and escutcheon.

of 4072 at 0.1 and 0.25%. Both were slightly more effective at 1 day and less effective at 1 week than the standard. At 2 weeks posttreatment, numbers of ticks approached or exceeded pretreatment levels.

WINTER TICKS.—All parasitic forms of the winter tick, a one-host species, are found on cattle in the vicinity of Kerrville during the months of November through February. Because of the winter tick's one-host cycle and seasonal incidence, a single treatment is usually sufficient to keep cattle free throughout the remainder of the tick season. It is important that an insecticide kill all feeding forms, and also have enough residual effectiveness to insure kill of newly molted nymphs and adults.

Methods used to evaluate General Chemical 3582 and 4072 against the winter tick were similar to those of Drummond et al. (1959). Portions of the dewlap and escutcheon of cows were examined for ticks, and the number of adults recorded before treatment, at 1 day, 1 week, and 1 month after treatment. Each treatment group of five cows was sprayed with one of the test insecticides or with 0.5% toxaphene. The results of these tests are summarized in table 2.

Table 2.—Effectiveness of General Chemical 3582 and 4072 as sprays against winter ticks on cattle, 1959–1961.

Insecticide	Concentration (%)	Average Number of Adult Ticks on 5 Cows[a]			
		Before Treatment	After Treatment		
			1 Day	1 Week	1 Month
		Sprayed December 10, 1959			
4072	0.25	>200	25.4	2.4	6.0
	.5	>200	10.4	1.4	2.2
Toxaphene	.5	>200	10.0	5.0	5.0
		Sprayed January 4, 1961			
3582	0.1	5.4	1.4	0	0
Toxaphene	.5	8.8	1.8	0	0
		Sprayed January 9, 1961			
3582	0.1	11.2	3.4	0	0
Toxaphene	.5	15.0	0.6	0	0

[a] On portions of dewlap and escutcheon.

Both the 0.25% and 0.5% concentrations of 4072 were effective in initial control of ticks. At 1 week both were superior to 0.5% toxaphene, and numbers of ticks were very low at 1 month. Sprays of 3582 were not so effective as toxaphene in initial control, but at 1 week and 1 month little difference existed in the protection afforded by the two treatments.

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Toxicity Studies of General Chemical 4072 and Two Related Compounds to Cattle[1]

R. D. RADELEFF, LAWANDA M. HUNT and CARL P. WEIDENBACH, *Animal Disease and Parasite Research Division, U.S.D.A., Kerrville, Texas*

General Chemical 4072(2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) has shown usefulness in entomological practice, as have two related materials, General Chemicals compounds 3582(1-(2,5-dichlorophenyl)-2-2-dichlorovinyl diethyl phosphate) and 3583(2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate). The three compounds are inhibitors of mammalism cholinesterase.

Preliminary toxicological evaluation of the three compounds was required before they were used as insecticides in the research

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Table 1.—Results of toxicity studies with three General Chemical Company compounds.

Animal	Age	Dosage (mg./kg.)	Number Treated	Poisoning		Unaffected	Cholinesterase Activity Lowest Reading (% of normal)
				Mild to Severe	Severe to Lethal		
			Compound 4072 (Oral)				
Calves	1–2 weeks	10	1	—	—	1	27
Cattle	1 year	10	2	—	—	2	52
		20	2	2	—	—	30
			Compound 4072 (Dermal)				
		(%)					
Calves	1–2 weeks	0.1	1	—	—	1	66
		0.25	1	—	—	1	66
		0.5	2	—	—	2	13
		1.0	1	—	—	1	13
		2.0	1	—	1	—	0
Cattle	1 year	0.1	4	—	—	4	85
		0.25	3	—	—	3	28
		0.5	24	7	1	16	23
			Compound 3582 (Dermal)				
Calves	1–2 weeks	0.1	1	—	—	1	29
		0.23	1	—	—	1	7
Cattle	1 year	0.1	1	—	—	1	76
		0.5	3	1	—	2	3
			Compound 3583 (Dermal)				
Calves	1–2 weeks	0.1	2	—	1	1	12
		0.25	1	—	1	—	31

program at Kerrville. The studies were not intended to be either extensive or complete, but to furnish guidance. (Table 1.)

The cattle and calves utilized in these studies were of various breeds, predominantly Hereford cattle and Holstein calves. All were in good health and free of complicating diseases. All were maintained and fed as are such animals in the Southwestern States.

The technical compounds were prepared as emulsifiable concentrates, using 25 parts of the compound, 65 parts xylene and 10 parts Triton X-100, by weight. These concentrates were prepared each day of treatment and were then mixed with water to obtain the desired final concentration for spraying. Commercial wettable powders were also freshly mixed with water before each spraying. The technical compounds were placed in gelatin capsules for oral administration.

In spray tests the animals were thoroughly wetted with approximately 1 gallon of liquid.

Jugular blood samples were drawn prior to treatment and at intervals, as indicated, following the treatment.

The treated animals were observed frequently throughout the day and, when required, at night.

Compound 4072.—Young dairy calves appeared to tolerate compound 4072 at more than 1% concentration when it was applied dermally. In the first few treatments of mature cattle (15 head) 0.5% concentrations were tolerated. In other research, Dr. R. O. Drummond of Entomology Research Division, treated five cattle with 0.5% sprays. The owner of the cattle was able to single out these five from other treated cattle without knowing the treatment groups, observing that the cattle lacked spirit. Additional treatments at Kerrville confirmed these observations and in addition produced salivation, dyspnea and muscular weakness.

Compound 3582.—Young dairy calves appeared to tolerate 0.1% sprays. A concentration of 0.25% produced almost complete depression of cholinesterase activity. One of three mature cattle sprayed with 0.5% concentration was poisoned.

Compound 3583.—This material was the most toxic of the three, producing poisoning of young dairy calves at 0.25% and 0.1% concentrations. Since a much higher concentration was indicated for insect control, no further work was done to develop toxicity data.

DISCUSSION.—From the limited data available, it would appear that compound 4072 should not be used on cattle at concentration greater than 0.25% and that this concentration could probably also be used on young calves. For oral administration 10 mg./kg. would appear to be the maximum permissible.

In the same manner a concentration of 0.1% to 0.25% of compound 3582 would appear to be the maximum safe dosage.

Compound 3583 appears to be toxic at the dosages required for control of animal parasites.

All three compounds induce salivation, dyspnea, muscular weakness, and diarrhea in animals given excessive dosages. These effects are effectively reversed by the administration of adequate amounts of atropine sulfate. Recovery from poisoning is usually rapid and complete.

Control of Lice on Livestock and Parasites on Poultry with General Chemical 4072[1]

R. A. HOFFMAN and R. O. DRUMMOND,[2] *Livestock Insects Investigations, Agric. Res. Serv., U.S.D.A., Stoneville, Mississippi*

Preliminary tests with General Chemical 4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) as an insecticide for cattle, goat, and poultry lice were conducted at Stoneville, Mississippi and Kerrville, Texas to establish levels of effectiveness for planning future field tests.

In tests against the sucking louse, *Solenoptes capillatus* Enderlein, pairs of heavily infested steer calves were sprayed with 2 quarts per head of 0.1% and 0.25% suspensions of the compound applied with a 2-gallon compressed-air sprayer.

[1] Accepted for publication July 12, 1961.

[2] Livestock Insects Laboratory, Kerrville, Texas.

Because this insect infests the animal's face, head, and brisket more heavily than other parts of the body, the front half of each steer was sprayed more thoroughly than the remainder of the animal. Louse counts, in numbers per square inch, were made at seven selected locations on each animal. Since heat will cause the lice to move to the ends of the hairs, counting was facilitated by directing an infrared heat lamp on the infested areas. The results indicated that 100% mortality of motile forms was obtained with both concentrations.

The effectiveness of General Chemical 4072 for control of the goat biting louse (*Bovicola caprae* (Gurlt)) was first demonstrated at Kerrville in a test in which two Angora goats were dipped in a 0.5% suspension of the toxicant. Complete initial kill of lice was obtained. Goats were maintained in an isolated pen and checked at intervals after treatment. No live lice were found through 51 days posttreatment.

In the summer of 1960, three ranch flocks containing 322 to 360 recently shorn Angora goats were sprayed with 0.05%, 0.1%, and 0.2% suspensions, respectively, of the compound. Sprays were applied with a power sprayer at a rate of approximately 1 quart per goat. At 24 hours posttreatment no live lice could be found on the goats. Ten goats from the flock treated at 0.2% were examined at 1 month after treatment and three live lice were found. At the next shearing, 6 months later, the flocks treated at 0.05% and 0.2% were examined, and the goats were lightly to heavily infested with lice. From these data, it is apparent that a single spraying with the indicated concentrations will afford excellent initial kill, but goats may be reinfested by the time of the next shearing. The reinfestation may have come from lice hatched from eggs laid on the goats before spraying, from lice on goats that received little or no spray, or from lice on flocks of neighboring ranches.

Effectiveness of General Chemical 4072 compares with that of 0.5% malathion. Smith & Richards (1955) reported that 24 hours after a single spraying with malathion no live lice were found, but at the next shearing (6 months later) the flock was lightly infested with lice.

The chicken body louse (*Menacanthus stramineus* (Nitzsch)) and the shaft louse (*Menopon gallinae* (L.)) were both present on the poultry used in tests with General Chemical 4072 at Stoneville. However, previous tests with several common toxicants had not shown any significant difference in susceptibility between the two species, and no attempt was made to distinguish between their susceptibility to 4072.

In these trials, each bird was sprayed individually with 40 milliliters of a 0.05% or 0.1% suspension applied with a small carbon dioxide-pressured hand-nozzle. Four birds were treated at each concentration. To determine mortality, before- and after-treatment counts of the total number of live lice per bird were made by opening the feathers at the vent, breast, back, neck, and each wing.

Examinations made 3 days after treatment indicated both concentrations had given approximately 80% reduction in the number of lice on the birds. At 5, 7, 10, 15, and 20 days, all the birds were completely free of lice; in these tests no difference was apparent in the effectiveness of the two concentrations. The results are comparable to those with malathion at similar concentrations (Hoffman 1960, 1961).

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Residues in the Milk of Dairy Cows Sprayed with P^{32}-Labeled General Chemical 4072[1]

R. H. ROBERTS,[2] R. D. RADELEFF,[3] and H. V. CLABORN,[2]
Agric. Res. Serv., U.S.D.A., Kerrville, Texas

The paucity of insecticides that can be used for control of insects affecting dairy cattle has emphasized the need for finding new ones that can be applied without contaminating milk. For this reason, new materials that are effective in laboratory and field tests are investigated to determine whether they will appear in the milk produced by treated cows.

A compound that has recently shown entomological effectiveness is General Chemical 4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate). Experiments were conducted with a P^{32}-labeled sample to study its secretion in the milk following its application to two dairy cows. Field-test data had indicated that the most probable use of 4072 in animal treatment would be as a spray, and therefore its presence in the milk was determined after dermal application.

The first animal, a Holstein, was treated with a water-base spray formulated from an emulsifiable concentrate containing 5 grams of P^{32}-labeled General Chemical 4072 and 15 grams of a solvent-emulsifier mixture containing 65 parts of xylene and 10 parts of Triton X-100.[4] Although this amount of insecticide was sufficient to make 2 liters of a 0.25% spray, the concentrate was diluted with water to 400 ml. A chromatography spray-bottle was used to apply 200 ml. to each side of the animal; all the body areas except the udder were covered. The spray solution was worked into the hair with a comb to insure penetration to the skin

The second animal, a Jersey, was also treated with 5 grams of P^{32}-labeled 4072, but the formulation was modified. The spray contained the chemical, 50 grams of xylene, with 5 grams of lanolin added as a sticker. The total volume of the prepared spray was about 60 ml. With a chromatography spray-bottle held 6 to 12 inches from the animal half of the material was applied on each side to all areas except the udder. Thus a fine mist was deposited on the outer hair cover; but the spray was not worked into the hair. About a 5% loss of material occurred during each treatment.

When received, the P^{32}-labeled compound had a specific activity of 3.4 millicuries per gram. The first animals was treated 1 day after, and the second, 16 days after receipt (when the compound had a specific activity of 1.7 millicuries per gram).

The weight of the milk and the percentage of butterfat were recorded for each sampling period. Samples for analysis were obtained by taking duplicate aliquots of 200 ml. from the morning milk just before treatment and at 5 hours and 1, 2, 3, 5, 7, 9, 10, and 12 days after treatment. The organosoluble components in the 200-ml. milk samples were extracted and purified by the method described by Claborn et al. (1960). The extract was concentrated to a volume of 5 ml. and an aliquot was measured radiometrically with an end-window Geiger-Müller tube.

The residue found, and the amount that would be present in milk with butterfat content of 4%, are presented in table 1. The greatest organosoluble residue occurred in the 5-hour samples. Thereafter the parts per million of General Chemical 4072 secreted gradually diminished and reached zero in the milk from the Holstein 12 days after and in that from the Jersey 10 days after treatment.

One interesting observation was that milk from the cow treated with the small volume of xylene-base spray misted on the outer hair contained only half or less of the residue found in the milk from the cow treated with the water-base spray. These data indicate that although the amount of technical material

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[2] Entomology Research Division.
[3] Animal Disease and Parasite Research Division.
[4] An alkylated aryl polyether alcohol.

Table 1.—Amount of milk produced, percentage of butterfat, and parts per million of organosoluble residue in milk of two dairy cows treated dermally with P^{32}-labeled General Chemical 4072.

Time Post-treatment	Pounds of Milk	Per Cent Butterfat	P.P.M. of 4072	
			Actually Found	Adjusted for 4% Butterfat
5 grams applied in 400 ml. of spray				
Control	25.5	2.4	—	—
5 hours	14.6	6.3	0.0584	0.0371
1 day	27.2	2.3	.0107	.0186
2 days	26.2	1.3	.0055	.0169
3 days	28.7	2.3	.0060	.0104
5 days	27.1	2.7	.0020	.0029
7 days	29.4	2.9	.0008	.0011
9 days	25.9	1.9	.0006	.0013
10 days	—	—	—	—
12 days	28.5	2.0	.0	.0
5 grams applied in 60 ml. of spray				
Control	19.8	2.5	—	—
5 hours	10.7	4.1	0.0228	0.0222
1 day	22.2	2.2	.0048	.0087
2 days	22.9	2.0	.0024	.0048
3 days	23.6	1.9	.0016	.0034
5 days	25.6	2.1	.0008	.0015
7 days	25.0	1.9	.0004	.0008
9 days	—	—	—	—
10 days	25.0	2.0	.0	.0

used on each animal was the same, absorption, as determined by the secretion levels, was greater when the spray was brought into contact with the skin.

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The Survival of Several Species of Insects in a Nitrogen Atmosphere[1]

GARY D. KNIPLING, W. N. SULLIVAN, and R. A. FULTON, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

To determine the maximum and minimum ratios of oxygen to nitrogen at which insects will survive, Sullivan et al. (1961) used the Madeira cockroach (*Leucophaea maderae* (F.)). They found that the insect died when the partial pressure of oxygen in an oxygen-nitrogen atmosphere was decreased to a point at which between 1% and 5% of oxygen was left. It was also observed (Fulton & Sullivan, unpublished data) that the cockroach could not survive when the carbon dioxide content of the atmosphere was greater than 26% even with an oxygen content greater than 4%.

Gilmour (1940) found that termites could not live in atmospheres of pure nitrogen for more than 7 hours. Other workers (Cotton & Young 1929, Cotton 1932) suggested the use of nitrogen and carbon dioxide to reduce fire hazard when flammable fumigants were involved or to increase the penetration of the gas into the tracheal system of insects. It seemed of interest to determine the survival time in nitrogen atmospheres of different insect species having a wide range of metabolic rates.

PROCEDURE.—The insects used in these tests were the Madeira cockroach (*Leucophaea maderae* (F.)), house fly (*Musca domestica* L.), yellow-fever mosquito (*Aedes aegypti* (L.)), Japanese beetle (*Popillia japonica* Newman), red-legged grasshopper (nymphs) (*Melanoplus femur-rubrum* (De Geer)), Mexican bean beetle (*Epilachna varivestis* Mulsant), and confused flour beetle (*Tribolium confusum* (Jacquelin du Val)). The Madeira cockroach, house fly, yellow fever mosquito, and confused flour beetle were laboratory reared, whereas the Japanese beetle, red-legged grasshopper, and Mexican bean beetle were field-collected the same day as the experiment. All of the insects were fed before the tests were begun, with their natural or accepted laboratory diets. The average number of insects used for each time period in each test was 30 for the mosquitoes, flies, flour beetles, and Japanese beetles; 10 for the bean beetles and grasshoppers; and 4 for the cockroach. Each test was replicated at least three times.

The insects were placed in 380-ml. or 500-ml. ground-glass-stoppered bottles. The air in the bottles was replaced with nitrogen by flushing each bottle with 100% nitrogen (water pumped) at a pressure ranging from 10 to 12 p.s.i. for 2 minutes. Although great care was taken, it is possible that a small amount of oxygen might have remained in the bottles because of incomplete flushing or by entry as the hose was slowly withdrawn. After the bottles were filled with nitrogen, they were placed in a constant-temperature darkroom at 72° F. The check insects were also held in the constant-temperature room in bottles with tulle cloth stretched across the open top.

Table 1.—The survival time of several species of insects in a nitrogen atmosphere.

Insect	Exposure Period (Hours.)	Per Cent Mortality[a]	
		1 Day	4 Days
Yellow-fever mosquito	0.25	0	—
	0.50	24	—
	1	6	—
	2	90	—
	4	100	—
House fly	2	0	—
	4	67	—
	8	100	—
Mexican bean beetle	8	15	75
	16	89	100
	24	56	100
Madeira cockroach	1	0	0
	4	0	19
	8	30	70
	16	88	88
	24	100	100
Red-legged grasshopper	8	0	43
	16	13	67
	20	27	80
	24	33	100
Japanese beetle	24	4	52
	48	8	68
	72	39	93
	96	92	100
Confused flour beetle	24	12	38[b]
	48	39	64[c]
	72	47	63[d]
	96	—	88
	120	100	100

[a] The controls of all species showed no mortality.
[b] 7-day count.
[c] 6-day count.
[d] 5-day count.

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At the end of the exposure period that varied from 15 minutes to as long as 5 days, the nitrogen was replaced with air, and the bottles containing the insects were returned to the constant-temperature room. No food was supplied to the insects at this time. Twenty-four hours after the exposure period and airing of the bottles, a careful count of the dead, moribund, and live insects was made. The dead insects were discarded, whereas the moribund and live insects were again placed in the constant-temperature room. The final count of the insects was made 4 days after airing. Except for flies and mosquitoes the 4-day count was made to measure the final effects of the nitrogen exposure. Only 1-day counts were made on the flies and mosquitoes because of some mortality in the controls held over 24 hours without food or water. The results of the tests are shown in table 1. The results were plotted on linear graph paper, and the exposure times (hours) required to kill 50% (LT-50) of the insects at the time of the final count were read from the graphs. These values were as follows:

Insect	*LT-50 (hours)*
Yellow-fever mosquito	1.5
House fly	3.5
Mexican bean beetle	5.5
Madeira cockroach	6.8
Red-legged grasshopper	10.2
Japanese beetle	22.8
Confused flour beetle	36.0

RESULTS AND DISCUSSION.—The yellow-fever mosquito was the quickest to die in an atmosphere of nitrogen. The LT-50, for this insect, was reached following 1.5 hours of exposure to nitrogen, and 100% mortality occurred following the 4-hour exposure. The house fly also had a short survival time following exposure to nitrogen with an LT-50 of 3.5 hours and a LT-100 of 8 hours. Perhaps the high metabolic rates of the fly and mosquito (Anon. 1956) led to a quicker depletion of the oxygen reserves in these insects. The confused flour beetle lived the longest, with an LT-50 of 36 hours of exposure to nitrogen.

Paralysis of the backlegs of the Japanese beetle and the Madeira cockroach was one of the most apparent effects of the exposure to nitrogen. The insects could walk, but their backlegs dragged along behind them. This effect was probably because of the lack of oxygen, since nitrogen does not have an effect on their nerve and muscle preparations (Frankel & Schneiderman 1958). In order to confirm that the air in the 380- and 500-ml. test bottles had been almost completely displaced by nitrogen, a check test was run with the Madeira cockroach. Several cockroaches were put in a separatory funnel, a vacuum was drawn, and nitrogen gas was then pumped until the pressure was back to 1 atmosphere. The mortality of the insects did not vary much from the average of the other tests in which the air was replaced with nitrogen by flushing.

During the experiments it was also noticed that when the bottles were being flushed with nitrogen, the insects were completely immobilized after 20 to 30 seconds. With the mosquito and house fly, recovery was apparently complete following the 15-minute exposure to nitrogen, and with the Madeira cockroach after the 1-hour exposure. Brooks (1957) showed that the German cockroach (*Blattella germanica* (L.)) died several days after being exposed to carbon dioxide for 2½ hours. It appears that nitrogen may be an excellent substitute for carbon dioxide in anesthetizing insects for laboratory handling. Comparative tests to determine the effect of various periods of exposure to nitrogen and to carbon dioxide employing several species of insects commonly used for laboratory investigations would be necessary to establish the relative value of the two gases for anesthetizing insects.

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Concrete-Insecticide Mixtures Toxic to Termites[1]

T. C. ALLEN, G. R. ESENTHER, and R. D. SHENEFELT[2]

The use of insecticides to protect buildings from termite damage requires the placement of a toxic barrier between the structural wood and the ground. Proper construction, installation of barriers, application of insecticides to soil and use of timber impregnated with chemicals are among the most effective measures to discourage or prevent infestations by these pests.

Observations on the behavior of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), in the infested area of Sheboygan, Wisconsin, showed that the concrete construction was often the pathway for shelter tubes between soil and wood. Insecticidal treatment of internal voids and cracks in concrete foundations is a standard method of preventing or controlliing termite infestations. If such concrete is fabricated with a toxic agent that would cause mortality of termites through contact action, the problem of preventing termite movement over the concrete or through crevices in foundations might be eliminated or reduced.

Late in the summer of 1960, dieldrin, an insecticide highly toxic to termites, was incorporated into cement mixtures. This insecticide is very stable even under highly alkaline conditions (Negherbon 1959).

Concrete blocks, approximately 3×5×5 inches in size, containing this insecticide were poured for evaluating contact toxicity to exposed termites. A wettable powder containing 75% dieldrin was added to the water used in the preparation of the concrete. Concentration of dieldrin in the cement mixtures was approximately 0.1% and 1.6% in the cement (w/w). Concrete blocks containing the insecticide were similar to the check blocks except for a darker gray color. One week after fabrication the block surfaces containing 0.1% dieldrin caused 100% mortality to *R. flavipes* workers exposed for a period of only 1 minute. Exposures of nasutes and workers of *Nasutitermes columbicus* (Holmgren) exposed 10 minutes to a surface of concrete block

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[2] Professor, Department of Entomology; former Research Assistant (now Entomologist U. S. Forest Products Laboratory, Madison, Wis.); and Professor of Entomology, University of Wisconsin, Madison, respectively.

containing 0.1% dieldrin resulted in 100% mortality.

Following a storage period of approximately 4 months at 80° F. and 97% relative humidity, concrete blocks containing the insecticide at the 1.6% level were still toxic to all the termites. A half-hour exposure to blocks with the 0.1% level of dieldrin did not kill the insects. Our study was initiated without knowledge of the work of Gay & Wetherly (1959) who very recently reported control of termites with dieldrin emulsions added to concrete mixtures.

In October 1960, concrete posts containing the two levels of dieldrin were set in the ground at three locations having different soil types to initiate a study of the persistence and movement of this insecticide through concrete exposed to weathering. The variation in surface toxicity to termites of concrete containing technical dieldrin in various formulations and solvents, and the movement of this insecticide within concrete and into soil adjacent to the concrete are now being studied.

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Control of the Apple Maggot and Plum Curculio with Soil Insecticides[1]

Earl R. Oatman and E. P. Lichtenstein[2]

Endrin was demonstrated to be a potentially effective control measure for the apple maggot, *Rhagoletis pomonella* (Walsh), as a soil insecticide in 1957–1958 (Oatman 1959). The results of one year's study showed that it gave 96% reduction over the check. Dieldrin, aldrin, and heptachlor were less effective, giving 60% to 66% reduction. The studies were continued through 1960 to evaluate the residual toxicity of the materials.

Methods and Materials.—The methods and materials for initiating and conducting the studies in 1957–1958 were described previously (Oatman 1959). Each emergence cage was stocked with 150 maggot-infested apples on August 15 in 1958 and 100 on the same date in 1959. Similar samples held in the insectary yielded 650 prepupae in 1958 and 473 in 1959. Based on this information, it was estimated that 2,600 and 1,892 maggots, respectively, were provided for each treatment. Old apples were removed after adult emergence prior to restocking the cages in 1958 and 1959. In 1960 they were removed just before adult emergence to eliminate the few maggots which had been found to pupate inside or between apples. The emerging adults were collected in 1959 and 1960 in the same manner as described for 1958 (Oatman 1959).

Apples infested with plum curculio, *Conotrachelus nenuphar* (Herbst), were added to each cage in early July of 1959 and 1960, and the adults were counted as they emerged.

Soil samples (Longue loam) were collected for residue studies in October, 1960, 3¼ years after applications of aldrin, dieldrin, endrin, and heptachlor to replicated turf plots (6′×6′) at 5.3 lbs. actual toxicant/acre (Oatman 1959). Each sample consisted of 16 cores (3″×1″) collected at random from underneath and around the emergence cages, making a total of four samples per treatment and check.

The amount of insecticide residues in each sample was determined by a bioassay procedure (Edwards et al. 1957). Three-day-old *Drosophila melanogastor* Meigen were exposed directly to the soil samples. Since a major part of aldrin applied to the soil is converted in time to dieldrin (Lichtenstein et al. 1960), the aldrin-treated soil samples were measured against a dieldrin standard. However, heptachlor-treated soils were measured against a heptachlor standard, since the formation of heptachlor-epoxide from heptachlor in soils is not so pronounced as is the epoxidation of aldrin (Lichtenstein & Schulz 1960).

Table 1.—Comparison of the residual effectiveness of soil insecticides applied at 5.3 pounds of toxicant per acre in 1957 for control of the apple maggot.

Treatment[a,b]	Number Adults Emerged			Per Cent Reduction Over Check		
	1958	1959	1960	1958	1959	1960
Endrin, EC	34	29	7	95.8	97.5	98.8
Dieldrin, EC	282[c]	451	202	65.6	61.6	65.1
Heptachlor, EC	327	844	403	60.1	28.2	30.4
Aldrin, EC	305	983	438	62.8	16.8	24.4
Untreated	819	1,175	579	—	—	—

[a] Randomized complete block design replicated four times.

[b] Equivalent amounts applied to sod July 15 in 1 gallon water per plot (36 sq. ft.) using a knapsack sprayer.

[c] Adult counts associated with same vertical lines are not significantly different at the 5% level (Duncan's Multiple Range Test—1955).

Table 2.—Insecticidal residues in turf plots 3¼ years after application to sod at 5.3 pounds toxicant per acre in July 1957 for control of the apple maggot.

Insecticide	Recovered Materials[a]	
	Pounds per 3-Inch Acre	Per Cent of Applied
Aldrin, EC	0.21±0.02	4.0
Heptachlor, EC	0.21±0.01	4.0
Dieldrin, EC	0.45±0.05	8.5
Endrin, EC	0.90±0.12	17.0

[a] Soil residue samples taken October 1960.

Results and Discussion.—All insecticidal treatments were significantly different from the check at the 5% level in 1958 (table 1). However, only endrin and dieldrin were significantly different from all treatments throughout the study. Endrin gave 96% to 99% and dieldrin 62% to 66% reduction over the check through 1960. Aldrin and heptachlor, however, dropped from 63% and 60% reduction in 1958 to 17% and 28%, respectively, in 1959, and showed essentially the same control in 1960. The per cent reduction in 1958 and 1959 was probably influenced by adults emerging from pupae which were inside or between apples. This was substantiated somewhat by the overall increased reduction for 1960 following removal of old apples from cages prior to adult amergence (table 1).

Plum curculio adults were recovered only from the check cages, thus indicating the high toxicity of the concentration of all toxicants to the larvae. Endrin has not been shown previously to be effective for control of the plum curculio as a soil treatment. Snapp (1960) reported poor control of the plum curculio with endrin granules at the equivalent rate of 2 pounds per acre in soil-box experiments in Georgia.

The results of the soil residue determinations are shown in table 2. The data correlated closely with the control data in table 1. Endrin was the most persistent insecticide and aldrin and heptachlor the least persistent. The results indicate that an annual application of endrin at 1½ to 2 pounds actual toxicant per acre might be sufficient for effective control of the apple maggot. Thus, the use of endrin, recommended for orchard mouse control at 1.4 to 2.4 pounds actual per acre, probably also helps control the apple maggot and plum curculio.

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[2] Assistant Professors in the Department of Entomology, University of Wisconsin, Madison.

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Further Tests of the Effectiveness of Cotton Stalk Shredders for Controlling the Pink Bollworm[1]

H. M. GRAHAM and O. T. ROBERTSON, *Entomology Research Division Agric. Res. Serv., U.S.D.A.*, and P. L. ADKISSON and L. H. WILKES, *Texas Agricultural Experiment Station*

The value of shredding cotton stalks and debris after harvest to reduce overwintering populations of the pink bollworm (*Pectinophora gossypiella* (Saunders)) has been described by Wilkes et al. (1959), Adkisson et al. (1960), and Chapman et al. (1961). These studies have emphasized the superiority of vertically rotating, flail machines over horizontally rotating, fixed-blade types. Schematic diagrams of these two types of machines are presented by Wilkes et al. (1959).

The tests reported herein were conducted to determine the effectiveness of recent developments and modifications made on flail and conventional horizontally rotating-blade shredders. The value of these modifications was measured by comparing their results with those obtained with a flail-type shredder used in previous tests reported by Wilkes et al. (1959). This machine (hereafter referred to as Machine A) had an effective swath width of 80 inches and employed 40 vertical free-swinging knives rotating on a horizontal shaft at 1620 revolutions per minute (r.p.m.). The second machine included in this test (Machine B) was a heretofore untested model of a flail-type shredder. It was similar to Machine A except the swath width was 60 inches and it employed 30 vertical free-swinging knives rotating on a horizontal shaft at 1750 r.p.m. Machine B also had a somewhat more rigid mounting assembly for the rotating knives. The third machine (Machine C) was a modified conventional horizontally rotating blade-type shredder. To provide additional shredding action with this type of machine, the modifications consisted of two pairs of rotating blades plus two stationary cutting edges mounted rigidly on the interior of the shredder housing. This modified machine was in contrast to previously tested horizontal machines that had only a single rotating blade with two cutting edges without stationary blades on the housing. The swath width of Machine C was 66 inches.

The tests were conducted in a heavily infested cotton field in the El Paso Valley, Texas, during October 1959. The field had been picked twice and about 80% of the remaining bolls were open. The plant growth was heavy, with the plants about 6 feet tall.

The experiment was laid out in a randomized-block arrangement with four replications. Each plot was four rows wide and approximately 50 feet long. Only the center two rows of each plot were used in the tests. The treatments, as indicated in table 1, consisted of shredding by the three machines at two speeds plus an unshredded check.

Table 1.—Emergence of pink bollworm moths following different stalk-shredder treatments (16 subplots).

MACHINE AND SPEED[a]	MEAN EMERGENCE[b]	REDUCTION FROM CHECK (%)
A		
Low	140.5 a	81.2
High	155.5 a	79.8
B		
Low	102.0 a	87.4
High	134.9 a[c]	82.5
C		
Low	334.6 b	56.5
High	363.6 b	52.8
Check	769.8	—

[a] Low = first gear (1.5 m.p.h.); high = third gear (3 m.p.h.).
[b] Means followed by the same letter are not significantly different at 5% level.
[c] Based on 12 subplots.

Following shredding, all debris containing cottonseed and lint was collected from each of four 1/1000-acre subsamples in each plot. In the unshredded check plots the samples consisted of all bolls found in similar subsample areas. These subsamples were held at Brownsville in large cans equipped with moth traps under conditions favorable for emergence of pink bollworm adults. The material was moistened occasionally to maintain favorable conditions until emergence was completed.

Records of the emergence of moths from the various treatments are presented in table 1. No significant differences were found between the two flail shredders, but both were significantly superior to the horizontal-blade machine. The reductions in emergence from the various shredding treatments were similar in magnitude to those reported previously. Reductions by the two flail shredders may have been exaggerated somewhat because some of the material was thrown from the plots by these shredders. No significant differences were found in operating the machines in high or low gear or between the interaction of machines and speeds. However, there was a trend toward lowered effectiveness with increase in travel speed. This trend was also noted in earlier tests.

The rate of emergence did not appear to be different from that

Table 2.—Total emergence per month of pink bollworm moths from stalk-shredder treatments.

MACHINE AND SPEED[a]	PER CENT OF MOTHS EMERGING IN—					
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
A						
Low	18.0	16.4	48.8	13.1	2.8	0.9
High	18.8	15.8	47.7	14.1	2.4	1.2
B						
Low	22.6	16.6	44.9	11.9	3.0	0.9
High	24.9	27.0	37.2	8.6	2.0	0.4
C						
Low	17.7	19.8	43.4	14.2	3.5	1.3
High	17.7	19.4	44.9	13.9	3.1	1.0
Check	13.4	19.3	46.9	14.4	4.2	1.5

[a] Low = first gear (1.5 m.p.h.); high = third gear (3 m.p.h.).

[1] Accepted for publication May 15, 1961.

of the check and treatments with Machines A and C (table 2). Emergence seemed to be somewhat more rapid from the material shredded by Machine B.

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Insecticide Residues on Red Clover After Clover Root Borer Control with Aldrin and Heptachlor Granules[1]

E. A. DICKASON and L. C. TERRIERE, *Oregon State University, Corvallis*

The clover root borer, *Hylastinus obscurus* (Marsham), is a major insect pest of second- and third-year stands of red clover. Marshall et al. (1949) and App & Everly (1950) first published on control of this insect with spray and dust applications of insecticides. Since that time, workers in both the United States and Canada have reported on chemical control of the clover root borer. Until recently, the standard control recommendation in Oregon has been 2 pounds of actual aldrin or heptachlor per acre applied as a dust during mid-April. Changes in the residue laws made it necessary to drop this recommendation since earlier residue studies, table 1, indicated that traces of residues would be present at harvest.

During 1960 an experiment was designed to evaluate insecticide performance and to determine chemical residues resulting from dormant season treatment. Granulated formulations of aldrin and heptachlor were tested on the basis of results obtained with these materials by Woodside & Turner (1956).

PROCEDURE.—During 1960, plots were established in a red clover field in its second seed-crop year at the Hyslop Agronomy Experimental Farm near Corvallis, Oregon. The insecticides were applied to soil and plant surfaces during the dormant season on February 24 in an attempt to control spring-migrating adults prior to their forming egg burrows and laying eggs in the crowns and roots of the red clover plants. A portion of the field was divided into nine 25- by 50-foot plots arranged in a randomized block design allowing three replications per treatment. A 5% granulated formulation of aldrin or heptachlor was applied at the rate of 14 ounces of actual toxicant per acre by means of a hand-pulled 3-foot-wide fertilizer spreader.

Plants were sampled for residue analyses and rate of growth immediately after application and at biweekly intervals until May 27. Samples were cut at a height of 1 inch above soil level from 10 "clumps" of clover from randomized areas throughout each plot and stored frozen in plastic bags.

Aldrin and dieldrin analyses were made according to Shell Method Series 3/59 and 4/59 (1959), respectively. Heptachlor and heptachlor epoxide were determined by a method supplied by Velsicol Chemical Corporation (1960). The reliability of these methods was established by the use of controls fortified with the respective insecticides with recoveries ranging from 75% to 100%.

The performance of chemicals on the clover root borer was evaluated during the fall of 1960 (approximately 7 months after treatment) by counting the number of plants and obtaining weight of green forage from five 3- by 3-foot predetermined randomized areas in each plot. Because of the high plant mortality in untreated plots, no attempt was made to score the roots for the number of insects present.

RESULTS AND DISCUSSION.—As shown in table 2, treatments with 14 ounces of actual insecticide per acre did not result in measurable harvesttime residues of aldrin or heptachlor or of their epoxide counterparts, dieldrin or heptachlor epoxide. Methods employed were sensitive to 0.03 p.p.m., aldrin or dieldrin; and 0.05 p.p.m., heptachlor or heptachlor epoxide. If translocation can be disregarded as a factor in this experiment, the observed growth dilution of approximately 100 times (sample weights increased from 30 to 2500 grams) indicates the maximum possible residues in these plots would be approximately 0.05 to 0.1 p.p.m. at harvest time. This would assume, however, that all of the pesticides present as a residue at treatment time would remain on the foliage through all weathering until harvest, 92 days later. In contrast to this dormant season treatment, aldrin or

Table 1.—Aldrin and heptachlor residues on red clover, 1952.

INSECTICIDE AND ACTIVE INGREDIENT PER ACRE (LB.)[a]	INTERVAL (DAYS)	RESIDUE (P.P.M.)[b]
Aldrin, 1.75 (2½% dust)	1	11.9
	8	2.6
	15	0.9
	29	0.3
	35	<0.2
Untreated	—	<0.2
Heptachlor, 1.83 (5% dust)	1	8.9
	8	1.1
	15	0.5
	29	0.2
	35	0.3
Untreated	—	<0.2

[a] Treated April 11, 1952, when plants were approximately 5 inches high.
[b] Treatments replicated three times, data are averages of three analyses. Samples analyzed by total chlorine method.

Table 2.—Aldrin, dieldrin, heptachlor, and heptachlor epoxide residues on red clover after early spring application of aldrin and heptachlor 5% granules, 1960.

INSECTICIDE AND ACTIVE INGREDIENT PER ACRE (OZ.)[a]	INTERVAL (DAYS)	RESIDUES (P.P.M.)[b]			
		Aldrin	Dieldrin	Heptachlor	Heptachlor Epoxide
Aldrin, 14	0	3.35			
	12	<0.25			
	26		<0.10		
	92	<0.03	<0.03		
Untreated	—	<0.03	<0.03		
Heptachlor, 14	0			7.95	
	12			0.23	0.15
	92			<0.05	<0.05
Untreated	—			<0.05	<0.05

[a] Treated February 24, 1960, when plants were approximately 2 inches high.
[b] Data represent an average of two or more analyses.

[1] Technical paper No. 1415, Oregon Agricultural Experiment Station. Accepted for publication May 16, 1961.

Table 3.—Effects of clover root borer control in red clover 7 months after treatment with aldrin or heptachlor granules, 1960.

INSECTICIDE AND ACTIVE INGREDIENT PER ACRE (OZ.)	STAND COUNT (NO. PLANTS)[a]	FORAGE YIELD (WT. IN GM.)[a]
Untreated	41	60.3
Aldrin, 14	154.3[b]	410.6[b]
Heptachlor, 14	185.7[b]	664.9[c]

[a] Average of three replications, five 3-×3-foot samples from each plot.
[b] Significant at 1% level, treated vs. untreated.
[c] Significant at 1% level, heptachlor vs. aldrin and untreated.

heptachlor dust applied during the growing season resulted in measurable residues at harvest time (table 1), 35 days later.

In a second experiment at Astoria, Oregon, heptachlor granules applied to dormant red clover on March 8, 1960, at the rate of 6 pounds of actual toxicant per acre resulted in a measurable residue of heptachlor and its epoxide at harvest time. Residues immediately after treatment averaged 49 p.p.m. of heptachlor, and 70 days later they averaged 0.05 p.p.m. of heptachlor and 0.07 p.p.m. of heptachlor epoxide.

A striking degree of pest control, as measured by stand count and yield of forage approximately 7 months after treatment, is indicated by the results shown in table 3.

The usual recommendation is to treat red clover for clover root borer control the spring following seeding, or the first seed crop year. Although this is the preferred treatment, the data in table 3 illustrate the effectiveness of a treatment (only treatment) the second seed-crop season. Similar results have been observed in Oregon where fields were treated for the first time the second crop year following seeding. Obviously, if a field were heavily infested with clover root borers, resulting in a high plant mortality, a delayed treatment would not be effective.

The studies reported herein indicate that an early-season treatment of red clover with aldrin or heptachlor granules at (approximately) 1 pound of actual toxicant per acre reduced plant mortality associated with clover root borer injury. It can also be concluded that such treatments will not result in measurable quantities of chemical residues at harvest.

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Effectiveness of Several Insecticidal Aerosols against the Japanese Beetle[1]

W. N. SULLIVAN, R. A. FULTON, GARY D. KNIPLING, and JULIAN C. RAINWATER,[2] *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Beltsville, Maryland*

During the summer of 1960 living Japanese beetles (*Popillia japonica* Newman) were found on jet aircraft arriving at non-infested west coast airports from infested areas in the eastern United States. They were also found in aircraft traveling from the eastern United States to foreign countries free of the Japanese beetles. This situation emphasized the need for improved aircraft disinsection. As part of a study of this problem, several new insecticidal aerosols were tested against the Japanese beetle.

It had previously been shown by Fulton et al. (1950), Hornstein et al. (1958), Ihndris & Sullivan (1958), and Yeomans et al. (1961) that vapors from organic phosphate insecticides were effective against insects.

Studies on the use of DDVP to prevent the spread of insects of public-health importance by aircraft travel have been made by Jensen et al., Pearce et al., and Hays (in press), who showed that vapors from a DDVP-dispensing device were very effective against flies and mosquitoes and there were no objectionable odors, irritating properties, or deleterious effects on aircraft construction materials.

Gaddis & Goodhue (1947) obtained 100% mortality of free-flying Japanese beetles when an aerosol formulation containing 3% DDT+0.4% pyrethrins was applied in aircraft at the rate of 7.5 grams of aerosol per 1,000 cubic feet. This or similar formulations have been used since that time to treat aircraft in preventing the spread of Japanese beetles. In the present work G-1152, the DDT-pyrethrum standard aerosol (3% DDT+1% pyrethrins) now in use, was compared with formulations containing Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), dimethoate, DDVP, allethrin, or barthrin.

The following general purpose (G) airplane aerosol formulations were tested:

Formulation	*Per Cent*
G-1428	
Dibrom	5.0
Hexylene Glycol[a]	5.0
Propellent 11 / Propellent 12 (1:1 W/W)	90.0
G-1447	
Dimethoate	5.0
Methyl chloride	95.0
G-1449	
DDVP	10.0
Methylene chloride / Propellent 12 (1:1 W/W)	90.0
G-1442	
Allethrin (90% pure)	5.55
Dimethoate	5.0
Methylene chloride / Propellent 12 (1:1 W/W)	89.45
G-1152	
Pyrethrum extract (20% pyrethrins)	5.0
DDT	3.0
Cyclohexanone	5.0
Lube oil	2.0
Propellent 11 / Propellent 12 (3:7 W/W)	85.0
G-1448	
Allethrin (90% pure)	1.1
DDT	2.0
Velsicol AR-60®[b]	6.0
Propellent 11 / Propellent 12 (1:1 W/W)	90.9
G-1445	
Allethrin (90% pure)	1.1
Barthrin	5.7
DDT	3.2
Cyclohexanone	5.0
Propellent 11 / Propellent 12 (3:7 W/W)	85.0
G-1365	
Allethrin (90% pure)	11.0
Propellent 11 / Propellent 12 (1:1 W/W)	89.0

[a] 2-Methyl-2,4-pentanediol.
[b] A methylated naphthalene hydrocarbon fraction.

From 100 to 200 beetles freshly collected in nature were liberated in 1,000-cu.-ft. test chambers at 20° to 26° C. 15 minutes prior to treatment. The test aerosols, packaged in 12-ounce cans with Model No. 1 Precision [3] valves, were applied through the chamber port at a weighed dosage and the insects were observed during a 30-minute exposure period. The beetles were then collected and placed in cages with sliced apples for food. Mortality counts were made after 1 and 4 days. The formulations and their concentration of active ingredients, dosage range, number of replications, and insect mortalities are given in table 1.

The dosage of DDT-pyrethrum required to kill the beetles in 1960 was far greater than that reported in 1947. This larger dosage suggests the possibility that Japanese beetles are becoming resistant to DDT. Dibrom, dimethoate, and DDVP were

[1] Accepted for publication May 16, 1961.
[2] Acknowledgement is made to M. S. Schechter for suggesting several of the formulations used in these tests.

[3] Use of this trade name does not necessarily imply endorsement of the product by the U. S. Department of Agriculture.

Table 1.—Effectiveness against Japanese beetles of several airplane aerosol formulations.[a]

Formulation and Concentration of Active Ingredients (mg./1,000 cu. ft.	No. of Tests	Mortality (%) in 1 Day	Mortality (%) in 4 Days	Formulation and Concentration of Active Ingredients (mg./1,000 cu. ft.	No. of Tests	Mortality (%) in 1 Day	Mortality (%) in 4 Days
G-1428				G-1152			
101	2	43	63	308	3	7	64
212	2	57	98	604	3	20	86
310	2	76	99	1,201	3	56	96
408	1	96	100	1,831	4	76	99
G-1447				G-1448			
102	3	30	69	465	1	9	63
203	3	42	96	947	1	—	100
407	3	76	97	1,365	1	61	95
				3,005	1	27	100
G-1449				G-1445			
107	3	39	52	749	2	14	51
206	3	48	72	1,135	3	18	78
401	3	72	99	1,496	3	11	73
801	3	85	100				
G-1442				G-1365			
200	2	—	75	531	2	0	21
310	2	—	97	1,020	2	0	31
810	2	—	95	1,519	3	0	47

[a] Field-collected beetles (100 to 200) exposed loose in 1,000-cu.-ft. chambers for one-half hour.

markedly superior to DDT-pyrethrum in killing the beetles. In most tests Dibrom and DDVP gave 100% knockdown of beetles in 20 to 30 minutes at the dosages used, but the knockdown by dimethoate was poor. Allethrin had a very effective knockdown action, but most of the insects recovered. Barthrin was not considered effective against the Japanese beetle.

From the present tests it would appear that DDVP as an aerosol is effective in controlling the spread of agricultural insects by aircraft. A portion of the DDVP aerosol volatilizes, thereby assuring greater penetration, and in combination with the aerosol particles gives better coverage throughout the aircraft. Practical use of DDVP, Dibrom, or dimethoate in aircraft disinsection will depend on toxicological data not yet available, and effects on aircraft components. Therefore at present none of the new formulations can be recommended for use in aircraft.

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Elm Spanworm Egg Mass Studies[1]

A. T. Drooz and J. D. Solomon, *Southeastern Forest Experiment Station, Forest Service, U.S.D.A., Asheville, North Carolina*

The elm spanworm, *Ennomos subsignarius* (Hübner), is a geometrid that has caused considerable loss of ridge-top oaks and hickories during an outbreak that has persisted in the southern Appalachians since 1954. This looper overwinters in the egg stage on the branches of host and even nonhost tree species. Therefore, egg mass samples may be used, with due regard for natural control, to predict the next season's spanworm activity. At the same time, certain characteristics of the masses are relevant to the whole egg mass sampling problem. These are: (1) oviposition host preference, (2) oviposition site preference on a branch, and (3) the number of eggs that constitute a mass.

In order to investigate this problem, analyses were undertaken of egg mass population data collected during an extensive survey in the tristate area of North Carolina, Georgia, and Tennessee, during September 1960. The samples were pruned from the lower half of the crown of red oaks (group), white oaks (group), and hickories. Although these are favored host species, they are by no means the only hosts fed upon. Two 5-foot branches were collected from two of each of the previously mentioned hosts. A total of 107 plots was established and 59 of these contained the predetermined host groupings (red oaks, white oaks, hickories).

A "t" test was applied to learn if there were significant differences between hosts for oviposition. As a result, no significance was found between red and white oaks, but significance at the 5% level was expressed between either oak group and the hickories (table 1).

When the data relative to where the eggs are deposited on the branch sample were analyzed, it was found that more than 60% were laid on sections between 0.4- and 0.6-inch in diameter; whereas only 14% were on sections 0.1- to 0.2- and 0.8- to 1.0-inch in diameter (fig. 1). Carrying this point farther, about 94% of the masses sampled were deposited between the second and fifth foot from the terminal bud. Therefore, as a matter of convenience one should have a good chance of taking an adequate

Table 1.—Comparison of the number of elm spanworm egg masses occurring on three different host groups (59 plots).

Host	No. Egg Masses	Mean Masses per Plot	Standard Deviation per Plot	Treatment	"t"
Red oaks	386	6.5	± 10.34	Red oaks vs. white oaks	0.425
White oaks	365	6.2	± 8.82	White oaks vs. hickories	2.278*
Hickories	237	4.0	± 5.36	Red oaks vs. hickories	2.401*

* Significant at the 5% level for 58 degrees of freedom where "t" = 2.000.

sample for survey purposes by cutting a 2-foot branch section, either from the third and fourth or fourth and fifth foot of the branch (fig. 2).

Examination of 1,421 egg masses from the three host groups showed that the mean number of eggs per mass was slightly above 70 for the oaks and 63 for the hickories (table 2). Although the variation of eggs per mass is rather high (the standard error of individual observations approaches the mean number of eggs per mass), the sampling of 300 or more masses yields estimates of the mean with reasonably small sampling errors.

It is concluded that no significant difference in the number of egg masses from red and white oaks was found. However, a significant difference in host preference was detected for the red and white oak groups over the hickories. The mean number of eggs per mass on the oaks tended to be greater than that for the hickories. Also greater numbers of the masses were found on the 0.4- to 0.6-inch-branch diameters. Few egg masses were located

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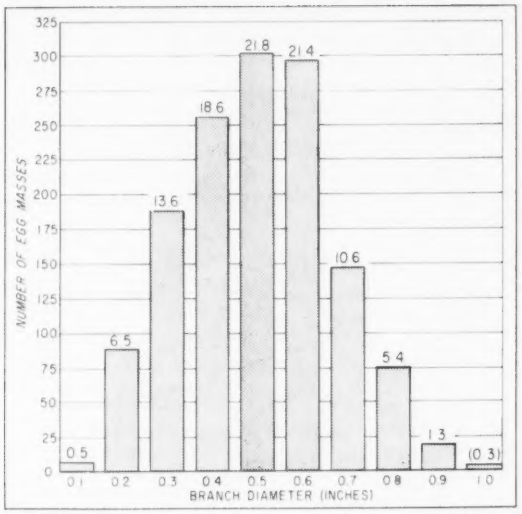

FIG. 1.—Elm spanworm egg masses by branch diameter. Numbers are per cent of total sample.

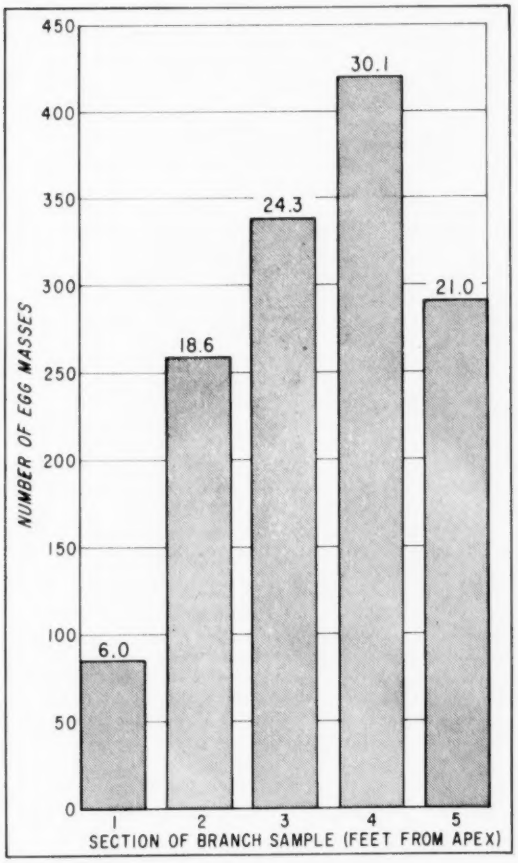

FIG. 2.—Elm spanworm egg masses by one-foot section of branch. Numbers are per cent of total sample.

Table 2.—Comparison of the number of elm spanworm eggs per mass by preferred host species.

HOST	NO. EGG MASSES	MEAN EGGS PER MASS ($\bar{x}$)	STANDARD ERROR OF MEAN EGGS PER MASS ($S_{\bar{x}}$)
Red oaks	620	70.60	±2.47
White oaks	500	71.87	±2.74
Hickories	301	63.00	±3.81

on the first foot of the branches from the terminal bud. As a practical measure, a 2-foot sample from the third and fourth or fourth and fifth foot should suffice for survey purposes.

Systemic Insecticidal Action of Certain Phosphates in *Pinus strobus* against *Diprion similis*[1]

HARRY C. COPPEL and DALE M. NORRIS, JR.,
University of Wisconsin, Madison

Previous organophosphate systemic research in both elm (Norris 1960) and white pine (Coppel & Norris 1960) indicated a consistent association of phosphate or phosphorothiolate molecules with the highest degrees of insect mortality, longest residual action throughout the tree and the only practical control of bark feeding. Results of additional studies of this nature in white pine are presented herein.

METHODS AND MATERIALS.—The general methods were as previously published unless specified (Coppel & Norris 1960). Three technical insecticides (phosphate molecules), Shell 3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate), Shell 1836 (*O,O*-diethyl 2-chlorovinyl phosphate) and phosphamidon, were implanted in white pines at 4, 5 or 8 grams actual per tree. The insecticides were known to be topically highly toxic to the introduced sawfly larvae (*Diprion similis* (Hartig)). Shell 3562, formulated as 50% actual by weight in two bark-penetrating agents, was painted on the basal trunk of white pines in 2-inch-wide bands; 20 g. actual per tree. All treatments were replicated on three or four trees.

Insecticidal action was measured by a bioassay method involving 25 third-instar sawfly larvae caged on a randomly selected terminal branch. Such assays were run at 3, 40, 364 and 406 days after treatment in Shell 3562-implanted trees and at 7, 21 and 63 days in the other studies. At each caging date, the bioassay was established on a branch in the lower, middle and upper crown of each tree. Larval mortality was checked at 24-hour intervals and all data represent death in 96 hours.

RESULTS AND DISCUSSION.—All implanted systemics gave practical levels of insect control (tables 1 and 3). Doubling the dosage from 4 to 8 g. increased the assayed practical residual effectiveness of Shell 3562 from 40 to 364 days (table 1). The actual practical residual of 4 g. was probably longer than 40 days, but the data still indicate that residual effectiveness can be increased with a larger initial dosage. The 8-g. quantity actually gave practical insect control for two growing seasons.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by the Wisconsin Conservation Department and the Shell Development Company and was reported at the Annual Meeting of the Entomological Society of America held at Atlantic City, New Jersey, November–December, 1960. Accepted for publication May 18, 1961.

Table 1.—Mean sawfly mortality in assays on white pine at various intervals after implantation with Shell 3562 during 1959 and 1960.

Actual per Tree (g.)	Per Cent Mean Mortality, Days After Treatment			
	3	40	364	406
4	81.0	100.0	35.3	16.8
8	77.0	100.0	83.9	52.7
Control	11.8	0.0	4.8	3.8

Table 2.—Mean sawfly mortality in assays on white pine at various intervals after treatment with 20 g. actual of Shell 3562 via bark painting, 1960.

Insecticide Formulations	Per Cent Mean Mortality, Days After Treatment		
	7	21	63
Shell 3562 (Code 3-2-55-2)	28.2	22.8	5.4
Shell 3562 (Code 3-2-54-2)	48.2	18.6	9.5
Control	5.8	4.2	3.8

Table 3.—Mean sawfly mortality in assays on white pine implanted with 5 g. actual of Shell 1836 or phosphamidon as related to days after treatment and crown position, 1960.

Crown Position	Per Cent Mean Mortality, Days After Treatment		
	7	21	63
Shell 1836			
Lower	68.0	68.0	46.7
Middle	75.7	72.3	77.0
Upper	100.0	100.0	98.0
Phosphamidon			
Lower	69.4	76.8	49.2
Middle	100.0	98.6	46.7
Upper	100.0	100.0	27.0
Control	5.8	4.2	3.8

Bark treatments of Shell 3562 at 20 g. actual did not give practical control (table 2). Higher dosages (especially of formulation Code 3-2-54-2) might provide such control.

Data for Shell 1836 and phosphamidon indicate an increase in insect mortality from lower to upper crown levels (table 3). This can be attributed to increasing uniformity of insecticidal distribution from lower to upper crown. Additional implantation sites should improve insect control in lower crown positions. Through 63 days after treatment, insect control in Shell 1836 trees changed significantly (95% level) only in the lower crown between 21 and 63 days. Phosphamidon was apparently less stable since significant changes occurred in all crown positions between 21 and 63 days. The extreme difference in the water solubility of these two chemicals may be involved in their relative stability in the tree.

No phytotoxicity was noted in the test trees. Studies to date strongly indicate that 2 to 3 years of practical sawfly control may be obtained with these compounds without causing phytotoxic symptoms.

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Clover Mite Control Studies[1]

Donald MacCreary and W. A. Connell,[2] *Delaware Agricultural Experiment Station, Newark*

The clover mite, *Bryobia praetiosa* Koch, was first reported from Delaware in 1931, but apparently the conditions needed to bring it to notice as an important household pest did not occur here until about 1947. Subsequent to that year, however, a rapidly increasing number of complaints of dwelling infestations were received. Consequently, studies on the habits and control of this species were initiated in 1955.

The habits of the clover mite have been reported in detail by English & Snetsinger (1957). Our observations on behavior substantiate their findings. This mite feeds on grasses and other low-growing plants and is common in lawns. Invasion of homes occurs accidentally by mites searching for oviposition sites. The latter normally are the trunks of rough-barked trees and similar surfaces. Movement between feeding sites and oviposition sites appears to be unoriented, so that once in a dwelling, the mites may move in any direction, rather than in the one providing the most direct route for return to the lawn. The increasingly common practice, particularly in new housing developments, of maintaining luxuriant lawns adjacent to the walls of dwellings has been an important factor contributing to the recent rise in invasions of homes by this mite.

Although a 2-foot-wide cultivated border outside the foundation of a home is an effective barrier against the clover mite, there is a demand for other types of control measures for temporary relief. Because of this, tests of chemicals for clover mite control were conducted. These trials were made around infested homes. In each case the outside of the walls of the building was treated, up to the level of the bottom of the first floor windows, together with an adjacent 10-foot-wide band of lawn. An examination was made for mites 24 hours and 1 week after treatment. Also, spring-treated homes were reexamined the following autumn and autumn-treated homes the following spring.

Three homes were treated with dusting sulfur. In one case the treatment was repeated by dusting the entire lawn 1 week after the first application. These treatments were not effective.

Malathion, 0.5 pint 57% emulsifiable per 5 gallons of water, was effective in three trials, but did not completely eliminate the infestations.

Genite EM-923® (2,4-dichlorophenyl ester of benzenesulfonic acid), 2.25 pints in 5 gallons of water, gave good control at the one location where it was used.

Kelthane® (1,1-bis(*p*-chlorophenyl)2,2,2-trichloroethanol), 0.25 pint 18% emulsifiable per 5 gallons of water was effective in six trials, completely eliminating the mites in each instance. However, reinfestation occurred in some cases by the following spring or fall. It was also effective in four trials in which the band of treated lawn was reduced to a 5-foot width. In the latter instances the spray was applied at an approximate rate of 2 gallons per 100 sq. ft. of lawn, whereas with the 10-foot wide bands

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[2] Professor and Associate Professor, respectively.

of Kelthane, and malathion and Genite as well, the application rate was 1 gallon per 100 sq. ft.

Although Kelthane was quite effective in preventing further invasion during a season, the homemaker's chief concern is with the horde already in the home. Several proprietary household insecticides were tried against these, with applications being made to baseboards, door jambs and window sills. One of these preparations, which rapidly cleaned up the inside-the-home portion of the infestation, was a residual-type aerosol containing 2% chlordane, 3% DDT, 10% orthodichlorobenzene and 15% petroleum distillate.

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Pavement Ant Attacking Sugar Beets in California[1]

W. HARRY LANGE, *University of California, Davis*

The pavement ant, *Tetramorium caespitum* (L.) (fig. 1), was first observed as a pest of sugar beets on May 8, 1953, near Clarksburg, Yolo County, California. No serious damage was recorded again until May, 1961, when two fields were damaged in the Woodland area—also in Yolo County.

Damage to sugar beets occurs when beets follow several years of alfalfa—particularly late-planted beets which are in a suitable stage for attack at a time when the ants are active.

In the 1953 attack the ants destroyed 20 acres of sugar beets by feeding on the germinating seeds and on the primary roots just below the crowns of the plants (fig. 2). This feeding often caused a complete girdling of the roots and subsequent death of the plants. Seedlings 3″ to 6″ high were often girdled. Ants fed on the sap exuded from the destroyed root area. Occasional damage was caused to the crowns and bases of the petioles.

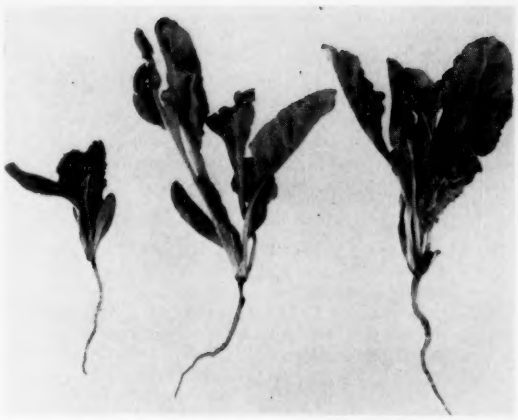

FIG. 2.—Damage of pavement ant to roots of sugar beet seedlings (healthy root on right).

On May 10, 1961, a field planted 3 weeks previously was found infested with the pavement ant in which the small seedlings were often cut off completely just below the soil level. Ants again were found feeding on the exuded sap. Ants in this instance were found to cause more damage at the periphery of the field and in areas through the field corresponding to the high check areas of the prior crop of alfalfa. In attacking, the ants usually concentrated on groups of plants in clumps moving then to adjacent groups of plants and killing all the plants in areas 10′ to 25′ long. The pattern of attack was definitely with the rows.

The pavement ant is found around the edges of sugar beet fields where they make flat mounds and often tend aphids—very

[1] Accepted for publication June 5, 1961.

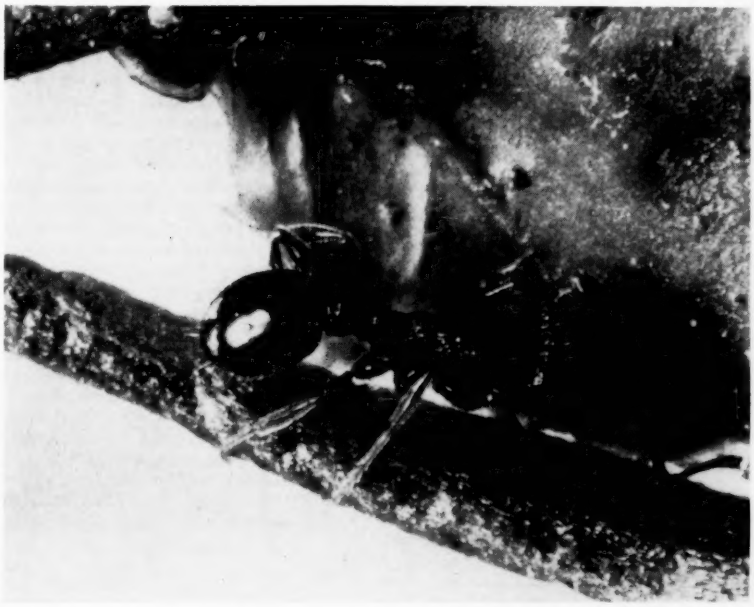

FIG. 1.—Pavement ant on leaf petiole of sugar beet.

often the bean aphid, *Aphis fabae* Scopoli, on *Rumex* spp., and other weeds.

The potential of the pavement ant as an agricultural pest was pointed out by Eckert & Mallis (1941), and Walker & Anderson (1937) recorded damage to eggplant.

Chlordane at the rate of 1 pound of active ingredient per acre, using 20 gallons of total spray per acre concentrated in the rows was found to give excellent control of the pavement ant attacking sugar beets.

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Walker, H. G., and **L. D. Anderson.** 1937. Control of the pavement ant attacking eggplants. Jour. Econ. Ent. 30: 312–4.

A Method for Estimating the Number of Spruce Budworm Eggs per Egg Mass[1]

JAMES L. BEAN[2]

The spruce budworm (*Choristoneura fumiferana* (Clemens)) has maintained an epidemic level in Minnesota since 1955. An intensive research program was initiated by the Lake States Forest Experiment Station, U. S. Forest Service, in 1956 to develop methods for controlling this pest. One of the problems encountered in this program was the estimation of egg deposition in the field.

In order to obtain a reliable estimate of egg parasitism, nonviability, and hatch, all egg-mass collections are taken after hatching has been completed. Viable eggs, before hatching, are easily distinguished in the egg mass and can be readily counted, but the boundaries of the translucent chorions of the hatched eggs are almost indistinguishable, making it extremely difficult to count the individual egg shells. Because of this, a study was undertaken to determine if the number of eggs per mass could be correlated with egg-mass length and/or width.

A sample of 45 unhatched egg masses from balsam fir in each of three different stand conditions was checked for the number of eggs per mass. As spruce budworm eggs are generally laid in 2- or 3-row masses, the data on eggs per mass were recorded for these two categories. These data were then subjected to a statistical analysis. The number of eggs per mass of given length varied similarly in each of the three conditions studied. A straight-line relationship was found between number of eggs and egg-mass length for egg masses of 3 mm. through 7 mm. in length. The extension of the regression line permitted the inclusion of data for egg-mass lengths for 2 mm. and for 8 mm. through 10 mm. Table 1 was developed from these data.

Table 1.—Estimated number of spruce budworm eggs per mass based on length of egg mass and number of rows of eggs.[a]

LENGTH OF MASS (MM.)	TWO ROWS OF EGGS	THREE ROWS OF EGGS
2	3.56	7.66
3	8.07	13.15
4	12.58	18.64
5	17.09	24.13
6	21.60	29.63
7	26.11	35.11
8	30.62	40.60
9	35.13	46.09
10	39.64	51.58

[a] This table can be used for egg masses collected either before or after hatching. Although the egg mass may contract somewhat in hatching, the original length is easily seen by marks left on the needle.

Further analysis of the raw data showed that egg-mass lengths ranged from 2 mm. to 10 mm., with approximately 64% equally distributed in the 2 mm. to 5 mm. range. The remainder was about equally distributed in the 6 mm. to 10 mm. range. This distribution pattern makes it undesirable to use an average number of eggs per mass for population studies.

This table was field tested the following year with 200 unhatched egg masses. A random set of 30 of these egg masses was subjected to a "t" test to determine if the average difference between estimated number, based on egg-mass length, and the actual number of eggs per mass was significant. This test led to the acceptance of the hypothesis that the mean difference between these two sets of numbers was equal to zero. Furthermore, these differences were not correlated with length of the egg mass.

In all our future studies of the spruce budworm in the Lake States, the number of spruce budworm eggs per egg mass will be estimated from length of egg mass as given in table 1.

[1] Accepted for publication June 7, 1961.

[2] Entomologist, Lake States Forest Experiment Station, Forest Service, U. S. Department of Agriculture. The Station is maintained at St. Paul 1, Minnesota, in cooperation with the University of Minnesota.

An Airflow Indicator to Aid in Fumigant Sampling[1]

M. MUTHU,[2] *Central Food Technological Research Institute, Mysore, India*

It is often difficult to know when the gas flow has ceased while fumigant samples are being drawn in evacuated flasks. This is especially true when the sampling is done through a packed bed of milled cereal products. Often the sampling tubes get choked, and the suction rate becomes very slow. These in turn contribute to erroneous results while expressing the gas concentrations, because of incomplete sampling of the fumigant. To aid in drawing a complete sample to satisfy the vacuum in the sampling flask, a simple air-flow indicator can be constructed and connected between the sampling point and the flask.

DESIGN OF THE AIRFLOW INDICATOR (Fig. 1.).—A glass tube of about 15 cm. in length is chosen and at one end, a 3-cm. length is bent at right angles over a Bunsen burner. After cooled, a plastic bead of slightly less diameter than the tube, so as to pass into the tube and have a free run inside, is inserted. Now the other end of the glass tube is similarly bent at right angles, but in the opposite direction to the first bend to form the shape of a Z. While making the bend, care should be taken to keep the bead away from the heat of the flame. After the tube has cooled, either a copper wire having a slight turned-in end or a plastic tubing with a wedge-shaped cut at the inserting end is thrust at either end, not only to imprison the bead but also to prevent its sticking at the ends during operation.

OPERATION.—The tube is placed in a vertical position. The top end of the tube is connected to the sampling flask with a length of pressure tubing and the lower end is connected to the sampling point.

On opening the stopcock of the evacuated flask, the bead gets sucked up and descends when the flow rate retards. Finally, it becomes stationary when the flow ceases. The last traces of flow may escape detection when the bead weight is more. To correct this, the indicator is tilted to a near horizontal position to allow the bead to move under lowered resistance, which will indicate the end point of suction and establishment of equilibrium between the fumigation chamber and the sampling flask.

The performance of the airflow indicator was tested with a

[1] Accepted for publication June 8, 1961.

[2] Junior Scientific Officer, Division of Infestation Control and Pesticides. The author gratefully acknowledges the kind interest shown by Dr. V. Subrahmanyan, Director, and Dr. A. Sreenivasan, Deputy Director, and thanks Mr. S. K. Majumder, Head of the Division, for suggestions given in this study.

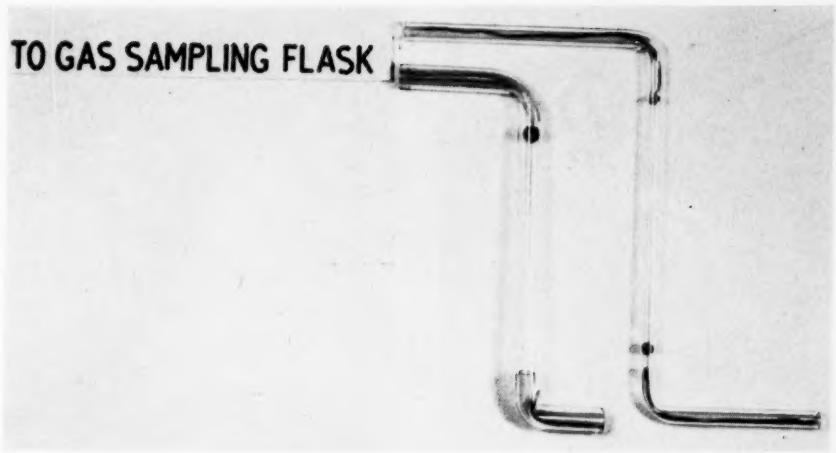

FIG. 1.—The airflow indicator.

Table 1.—Angle of tilt vs. sensitivity to airflow.

MATERIAL	WEIGHT OF BEAD (MG.)	DIAMETER OF BEAD (MM.)	DIAMETER OF TUBE (MM.)	PERFORMANCE	
				Degree of Tilt	Flow (cc/min.)
Plastic	21	3	4	90[a]	235
				20	148
				10	75
				5	Not sensitive to 30
Glass	25	2	3	90[a]	235
				15	148
				10	75
				5	30

[a] Normal position.

plastic bead (the holes being closed with plasticene) of 3 mm. diameter weighing 21 mg. in a 10-cm. length of glass tubing of 4 mm. diameter, having a run of 6 cm. for the bead during operation. With a standard flowmeter, it was found to be sensitive to a rate of flow of 235 cc. of air per minute (3.9 cc./second) at 14.7 p.s.i. air pressure at an angle of 90°. The sensitivity was increased to a flow rate of 75 cc. per minute with a tilt of 10°. The flow rates recorded at different angles of tilt with the plastic and a glass bead are shown in table 1. The glass bead was more sensitive to the lower rate of flow, viz., 30 cc./minute, than the plastic bead, attributable to the surface characteristics of the two materials, rather than their comparative weights.

The "gadget" can be used for taking samples at atmospheric pressure with a Strand flask, and also to indicate restoration of atmospheric pressure in fumigation chambers in vacuum fumigation techniques.

Ants Attacking Fleas in Puerto Rico[1]

IRVING FOX[2] and ILEANA GARCIA-MOLL, *School of Medicine-School of Tropical Medicine, University of Puerto Rico, San Juan*

It is well known that certain ants are beneficial in that they prey on noxious insects, and in a few instances they have even been used deliberately for the biological control of pests. In Puerto Rico they are reported to be reducers of populations of the sugarcane borer, *Diatraea saccharalis* (F.) by Wolcott & Martorell (1937) and of house flies (*Musca domestica* L.) by Pimentel (1955). The incident discussed below indicates that they may also be of benefit against fleas.

A laboratory colony of the oriental rat flea, *Xenopsylla cheopis* (Rothschild) in the main building of the School of Medicine was found to be infested with ants on May 16, 1961. The ants could be seen with the naked eye attacking fleas viciously. To confirm this observation one ant was placed in a vial with two living adult fleas and observed under the stereoscopic microscope. Immediately the ant attacked a flea (fig. 1) and within 2 hours it had killed them both. Two flea larvae and two ants were placed in another vial and again in about the same time the ants had killed the larvae (fig. 2). In another experiment, one ant placed in a vial with one larva within 1 minute grasped the larva by the terminal segments, moving up the body as the larva struggled but not letting go of it. Within 10 minutes after thrashing about the larva was moribund. The ant let go of it for a few seconds and then grasped it with the mandibles again and appeared to suck out body fluids. The flea larva was dead and dry within 30 minutes.

Specimens of the ant were submitted to the U. S. Department of Agriculture where Dr. M. R. Smith determined the species as *Paratrechina longicornis* (Latreille) or the crazy ant known in Puerto Rico as *hormiga loca*. Smith (1936) reported that this species is one of the most important house-infesting ants on the Island and also occurs on sandy beaches and dry pastures as well as in moist and shaded areas. Pimentel (1955) found that the crazy ant will kill larvae and adult house flies, but it was not so important in this respect as the fire ant, *Solenopsis geminata* (F.). However, in our opinion, the crazy ants would be of more value against fleas than fire ants because they do not sting and therefore would be less likely to disturb the rat host, unlike the fire ants which are capable of killing young rats.

In Puerto Rico the reduction in the incidence of murine typhus fever in the absence of designed insecticidal rat flea control has been the subject of investigations for a number of years (Fox 1956, Fox & Garcia-Moll 1961). Rat ectoparasite surveys involving 2,943 domestic rats captured alive in the city of San

[1] Supported in part by Research Grant E-2887 from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service. Accepted for publication June 22, 1961.

[2] Mr. Francisco Romero-Alemán assisted with the observations reported in this paper.

FIG. 1.—The crazy ant ("hormiga loca"), *Paratrechina longicornis*, attacking a rat flea adult, *Xenopsylla cheopis*, in Puerto Rico.

FIG. 2.—The crazy ant ("hormiga loca"), *Paratrechina longicornis*, attacking a rat flea larva, *Xenopsylla cheopis*, in Puerto Rico.

Juan, Puerto Rico, from December 7, 1956, to May 22, 1961, showed that 43% of the traps which caught rats also contained ants, but they were not identified to species. Predation by the ants on flea larvae and adults in the nests of the rat hosts must now also be considered among the possible ecological factors responsible for the decimation of the rat flea population.

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Designation of Insecticides by Trademarked Names or Code Numbers In Society Publications

CARROLL N. SMITH, *Chairman, Committee on Insecticide Terminology*

Insecticides for which no approved common names are available may be designated by trademarked names or code numbers in publications of the Entomological Society of America, provided the compounds are also identified by proper chemical names. Except in unusual circumstances compounds should be identified by chemical names which follow the principles of nomenclature established by the American Chemical Society for use in Chemical Abstracts. Insecticides sold on the open market should be identified by the chemical names approved for use on the labels. For the convenience of authors and editors the Committee on Insecticide Terminology has prepared the following list of chemical names (as of July 18, 1961) to be used in the Society's publications to identify the insecticides without common names which are most frequently mentioned. The proprietary designations under which they are distributed are given in the first column of the list. These designations are protected by trademarks, and their publication here is not to be construed as a release for use as common names.

CHEMICAL NAMES TO BE USED WITH PROPRIETARY DESIGNATIONS

(Single hyphens should be used as shown; double hyphens indicate that words should be closed up if written consecutively)

PROPRIETARY DESIGNATION	CHEMICAL NAME	OTHER DESIGNATIONS THAT HAVE BEEN USED
American Cyanamid 24055	1,1-dimethyl-3-(*p*-acetamidophenyl)triazene	
Aramite	2-(*p-tert*-butylphenoxy) isopropyl 2-chloroethyl sulfite	compound 88R
Bayer 22408	*O,O*-diethyl *O*-naphthalimido phosphorothioate	
Bayer 25141	*O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothioate	
Bayer 25198	*O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothioate	
Bayer 29493	*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate	Baytex; S 1752; Lebaycid; Entex

PROPRIETARY DESIGNATION	CHEMICAL NAME	OTHER DESIGNATIONS THAT HAVE BEEN USED
Bayer 37344	4-(methylthio)-3,5-xylyl methylcarbamate	
Bayer 39007	*o*-isopropoxyphenyl methylcarbamate	
Chlorobenzilate	ethyl 4,4′-dichlorobenzilate	Geigy 338; G-23992
Co-ral	*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphorothioate	Bayer 21/199
Delnav	a mixture of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (70%) and related compounds	Hercules AC-528
Diazinon	*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	G-24480
Dibrom	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	RE-4355
Dilan	a mixture of 1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane (Prolan) and 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane (Bulan)	CS-708
Dimetilan	3-methyl-5-pyrazolyl dimethylcarbamate	G-22870
Dimite	4,4′-dichloro-*alpha*-methylbenzhydrol	DMC
Dipterex	(see Dylox)	
Di-Syston	*O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate	Bayer 19639
Dylox	*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate	Bayer L 13/59; Dipterex
Eradex	2,3-quinoxalinedithiol cyclic trithiocarbonate	Bayer 30686
Genite 923	2,4-dichlorophenyl benzenesulfonate	General Chemical 923; Genitol 923
ethyl Guthion	*O,O*-diethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate	Bayer 16259
Guthion	*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate	Bayer 17147; Gusathion
Indalone	butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate	butopyronoxyl
Isolan	1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate	G-23611
Karathane	a mixture of dinitro (1-methyl heptyl)phenyl crotonate (78%) and dinitro (1-methyl heptyl) phenol and related compounds (22%)	
Kelthane	1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol	Rohm & Haas FW-293
Kepone	decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one	General Chemical 1189
Lethane 60	*beta*-thiocyanoethyl esters of aliphatic acids containing 10 to 18 carbon atoms	thiocyanoethyl laurate
Lethane 384	*beta*-butoxy-*beta*′-thiocyanodiethyl ether	butoxy thiocyanodiethyl ether
Meta-Systox-R	*O,O*-dimethyl *S*-2-(ethylsulfinyl)ethyl phosphorothioate	Bayer 21907
Methyl Trithion	*O,O*-dimethyl *S*-*p*-chlorophenylthiomethyl phosphorodithioate	
MGK 264	*N*-(2-ethylhexyl)bicyclo[2.2.1]-hept-5-ene-2,3-dicarboximide	Octacide 264; Van Dyk 264
Neotran	bis(*p*-chlorophenoxy)methane	K-1875; Oxythane; DCPM
Perthane	a mixture of diethyl diphenyl dichloroethane (95%) and related reaction products (5%)	Q-137
Phosdrin	a mixture of the *alpha* isomer of 2-carbomethoxy-1-methylvinyl dimethyl phosphate (not less than 60%) and related compounds (not more than 40%)	Shell OS-2046
Phostex	a mixture of bis(dialkyloxyphosphinothioyl) disulfides (alkyl ratio 75% ethyl, 25% isopropyl)	Niagara 1137
Pirazinon	*O,O*-diethyl *O*-(2-propyl-4-methyl-6-pyrimidinyl) phosphorothioate	G-24622
Potasan	*O,O*-diethyl *O*-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphorothioate	E-838
Pyrazoxon	diethyl 3-methyl-5-pyrazolyl phosphate	G-24483
Pyrolan	3-methyl-1-phenyl-5-pyrazolyl dimethylcarbamate	G-22008
Ruelene	*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate	Dowco 132
Sevin	1-naphthyl *N*-methylcarbamate	Union Carbide 7744
Strobane	terpene polychlorinates (65% chlorine)	compound 3961
Sulphenone	*p*-chlorophenyl phenyl sulfone	R-242
Tedion	2,4,4′,5-tetrachlorodiphenyl sulfone	
Thanite	a mixture of isobornyl thiocyanoacetate (82%) and related compounds	terpinyl thiocyanoacetate
Thiodan	6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	Niagara 5462
Trithion	*O,O*-diethyl *S*-*p*-chlorophenylthiomethyl phosphorodithioate	Stauffer R-1303
V-C 13 Nemacide	*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate	V-C 1–13
Zectran	4-dimethylamino-3,5-xylyl methylcarbamate	
Zytron	*O*-(2,4-dichlorophenyl) *O*-methyl isopropyl phosphoramidothioate	

EDITORIAL

An Important Russian Entomological Journal

Entomologicheskoe Obozrenie, journal of the Russian All-Union Entomological Society, is now in its fortieth volume. Published in the Russian language, its contents have not been readily available to most workers outside the Russian orbit. In 1958, however, the translation of this journal into English was included in a project of the American Institute of Biological Sciences, financed by a grant from the National Science Foundation; and there are now available complete, dependable translations of volumes 37, 38 and 39, under the title *Entomological Review*. The current volume is being translated without undue delay as soon as it appears (the journal is a quarterly). Members of the Society who are not familiar with *Entomological Review* will be interested to know that it covers the whole field of entomology. For example, the last complete volume (vol. 39, comprising 737 pages in the translated edition) contains papers on the biology and control of various insect pests; on the ecology of certain pest groups; on the biological control of some species, including such widely distributed pests as the European corn borer and the diamondback moth; on insect morphology, histology and physiology; on experiments to determine the effects of radiation on certain species; and on geographical distribution and taxonomy. For taxonomists, indeed, the publication is indispensable. Volume 39 includes numerous papers in this field, which contain original descriptions of 19 new genera and 115 new species representing 11 different orders. In addition, there are new combinations of scientific names, new synonyms, keys, first descriptions of the opposite sex of various species previously known only from either the male or the female, redescriptions of little known species, and solid contributions to the knowledge of larval classification of certain groups. A publication of such scope and significance should not be ignored, especially since it is now available in translated form. Information concerning distribution and cost may be obtained from Subscriptions, American Institute of Biological Sciences, 2000 P Street, N. W., Washington 6, D. C.

C. F. W. Muesebeck
U. S. National Museum

BOOK REVIEW

Specifications for Pesticides—Insecticides—Rodenticides—Molluscicides—Herbicides—Auxiliary Chemicals—Spraying and Dusting Apparatus. Published by World Health Organization, Geneva, Second edition, 1961. Distributed through the Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York, 523 pages. $10.00.

This book contains specifications established by WHO Expert Committees for the pesticides and application equipment used by WHO in the control of human diseases. Sections are devoted to Insecticides, Rodenticides, Molluscicides, Herbicides, Auxiliary Chemicals, Spraying and Dusting Apparatus, plus an Annex giving detailed procedures of analysis common to several specifications. The introduction states, "A specification is almost always a compromise between what industry can make, what the user wants, and what he is prepared to pay for the product." In addition to this a compromise had to be reached between the views of the scientists from the many nations involved. In view of all this it is indeed a pleasure to report that these specifications are practical documents that can be used throughout the world to insure the procurement of effective pesticide formulations. Detailed chemical and physical standards have been established for the technical grade of each of the pesticidal chemicals used. In addition, formulations are specified for many of these chemicals.

The insecticides covered are DDT, BHC, methoxychlor, chlordane, dieldrin, pyrethrum, allethrin, Diazinon®, malathion, parathion, Chlorthion®, and Dipterex®. Specifications are given for most of these formulated as water-dispersible powders, emulsion concentrate (it is regrettable that the more accurate term "emulsifiable concentrate" is not used) and dusting-powders.

The section on rodenticides contains specifications for the technical products ANTU, arsenious oxide, red squill, zinc phosphide, Coumachlor®, warfarin, Fumarin®, and Pival®. Requirements for the last four of these are given for concentrates and water-soluble powders.

The molluscicides section is essentially the same as in the first first edition and covers copper sulfate, pentachlorophenol and its sodium salt.

The second edition contains a new section on herbicides. Simazine, 2,4-D, 2,4,5-T and 4-chloro-2-methylphenoxyacetic acid are covered as technical products. The acidic herbicides are also specified as sodium and amine salts and as esters.

Sucrose, sodium benzoate and tetrasodium ethylenediamine tetraacetate have been added to the Auxiliary Chemicals section. No changes have been made in the section on spraying and dusting apparatus.

A series of annexes that group together those analytic procedures and routine test methods that are common to several specifications conclude the book.

This book, together with the U. S. Federal Specifications for pesticides, from which much of the material was derived, constitutes the bulk of the publicly available specification information. Those concerned with the purchase of pesticide chemicals for control programs should find this book very useful. An effort has apparently been made to keep the analytical and test methods as simple as possible so that they can be carried out in a greater number of laboratories. This may reduce the accuracy and reproducibility obtained with the more sophisticated methods.

French and Spanish editions of this publication are scheduled to appear during 1961.

Elmer E. Fleck,
Entomology Research Division,
U. S. Department of Agriculture

OBITUARY

Stanley Barron Freeborn

1891–1960

Stanley Freeborn was born in Hudson, Massachusetts on December 11, 1891 and died at Davis, California on July 17, 1960. He received his Bachelor of Science degree in 1914 from Massachusetts Agricultural College where he gained inspiration in studies of Diptera and insect morphology under Professors Fernald and Crampton. During his senior year he also served as Deputy State Nursery Inspector.

In 1914 he was appointed instructor in Entomology at the University of California and was advanced to Assistant Professor in 1918, Associate Professor in 1925, and Professor in 1932. His first assignment was to assist Professor W. B. Herms in a mosquito survey of California. During World War I he served in the Medical Corps of the U. S. Army with malaria control and delousing duties at the Port of Embarkation at Newport News, Virginia. At the close of the war, through his association with Dr. L. L. Williams and Henry Rose Carter, he accepted a reserve commission in the U. S. Public Health Service. This was the beginning of a lifetime affiliation with this branch of government service.

Returning to California in 1919, he resumed teaching and research and completed the work on "Mosquitoes of California," for which he was awarded the Ph.D. at Amherst in 1924. In this same year he assumed administrative responsibility for Entomology and Parasitology on the Davis campus. At Davis Freeborn set

the full pattern of his diverse and productive career. He taught his favorite course in beginning entomology and his hobby, insect morphology, commuting to Berkeley twice a week to give these lectures. He also taught medical and veterinary entomology and was active in research on helminth parasites of poultry and sheep, fly control in dairy barns, corn ear worm, honey diastase, and mosquito biology and control. The human qualities of teacher, counselor, and friend endeared him to students and colleagues alike. In 1930 he worked on citrus insect pests in the Mediterranean area, with partial support of the California Spray-Chemical Corp.

In 1935 Freeborn became assistant to the dean of the statewide College of Agriculture and, two years later, assistant dean and Assistant Director of the Experiment Station. With this transfer to Berkeley he began a distinguished career as university administrator, although he continued his teaching and his special interest in mosquitoes.

During World War II, from 1942 to 1945, he was called to active duty in the Public Health Service as Senior Surgeon (Lt. Col.) in charge of operations for the program—Malaria Control in War Areas. With headquarters in Atlanta, Georgia, Freeborn ranged over the entire field of malaria, dengue and typhus control in the United States and its possessions. In the military as in private life he administered with kindness, humility and an informality that must be unique in military annals. He was one of the first to see the potentialities of the new insecticide, DDT, and it was largely on his advice that the whole program for the eradication of malaria was undertaken.

In 1945, Freeborn resumed his teaching and administrative duties at Berkeley. He also served as faculty representative on the Pacific Coast Conference and, in 1946, as president of the conference. This activity was an outgrowth of his lifelong interest in athletics and his service as an official at footgall games at Davis and Berkeley.

In 1952 he was named Provost of the Davis campus and, in 1958, Chancellor. During 1958 he went to Iran as a member of a team of American educators to make a survey of Tehran's teaching program. He received the honorary Doctor of Laws from the University of California at the Davis Commencement in June, 1959, just before his retirement. Previously, in 1949, he had been granted an honorary Doctor of Science by his alma mater, Massachusetts Agricultural College.

Freeborn's affiliation with the old Entomological Society of America dated from 1923, and he joined the American Association of Economic Entomologists in 1919. He took time from his heavy schedule to attend national and branch meetings, serving as Chairman of the Pacific Branch at its Second Annual meeting at Bend, Oregon in 1954. He was interested in the section on teaching and participated in symposia on this subject at national meetings. He served as consultant to the California Mosquito Abatement Districts even after retirement and had assumed a leading role in the Division of Research Grants of the U. S. Public Health Service at the time of his death.

The publications which he regarded most highly were his "Mosquitoes of California" (1926) and the recent California Insect Survey Bulletin with the same title (co-authored with R. M. Bohart), and his chapter on "Anophelines of the Nearctic Region" in Boyd's "Malariology." He was also proud of his role in developing pictorial keys and circular keys, which he referred to as "horrorscopes," to aid non-entomologists in the field identification of mosquitoes.

Dr. Freeborn's first wife, Mary Chase Freeborn, succumbed to cancer in 1946 and his second wife, Marion Gay Maclise Freeborn, died of the same affliction in 1959. The surviving members of his family are his son, Captain Stanley B. Freeborn, Jr., U.S.N., at Point Magu, California; his daughter, Mrs. Hans J. Jensen (Joyce), whose husband is with the U. S. Diplomatic Service at Oslo, Norway; and three grandchildren, Andrea, Peter, and Kurt Jensen.

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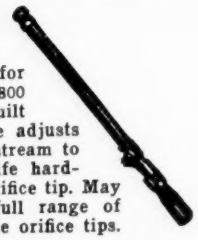

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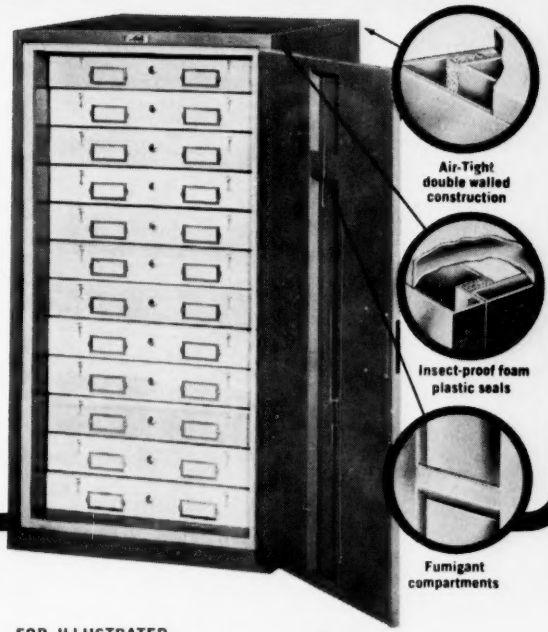

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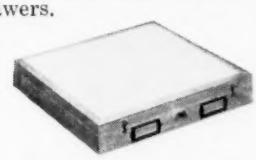

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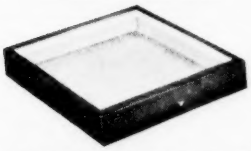

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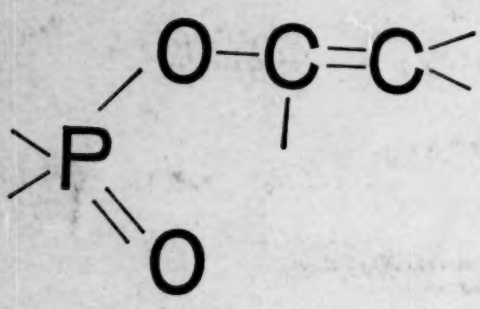

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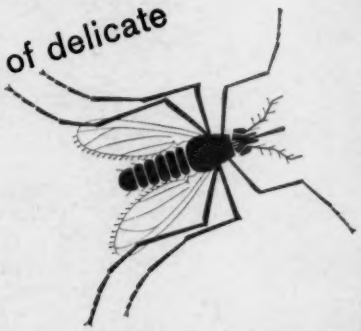

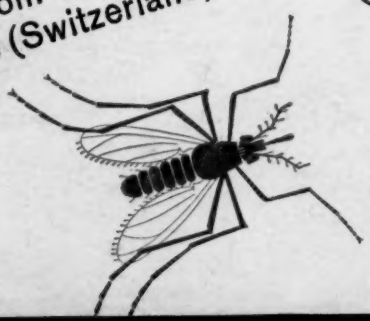

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